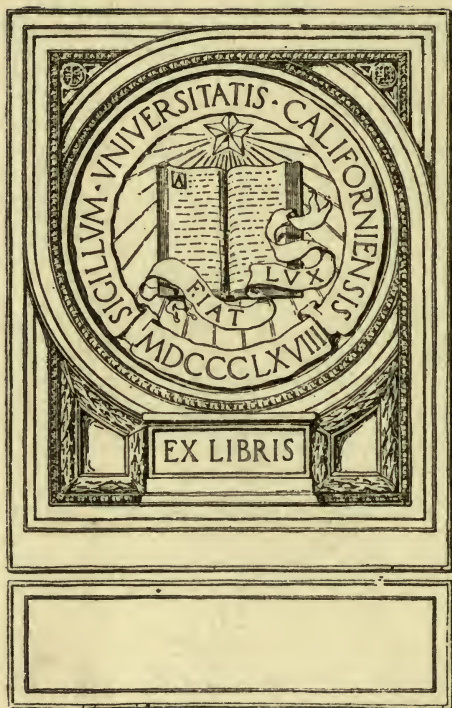


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A LABORATORY GUIDE
in
PHARMACOLOGY

By
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ILLUSTRATED



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W. B. SAUNDERS COMPANY
1917

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PREFACE

THE following exercises are designed to introduce the student personally to some of the more important facts of pharmacology. They have been selected so as to present little difficulty to one versed in ordinary chemic and physiologic technic, and require but little help on the part of the instructor.

The pharmaceutic and toxicologic exercises (Part I) are confined strictly to the bare essentials needed by students who intend to become general practitioners of medicine. Especial stress has been laid on the facts which have a direct practical bearing.

The experiments on animals (Part II) have been arranged in groups, to illustrate various types or phenomena, to bring out the similarities and differences of the response of organs to pharmacologic agents, rather than by individual drugs. This arrangement articulates better with the student's experience in physiology and pathology, on which pharmacology is largely founded. It is, therefore, more natural, as well as more interesting and inspiring. It has been the practice of the author to have this experimental course precede the didactic course. The latter, dealing with individual drugs, can then be based upon phenomena with which the student is already familiar.

The exercises are arranged for a course of thirty working periods of two to three hours. Additional experiments, for longer courses, demonstrations, etc., are introduced as optional. They can, of course, be indefinitely extended by the use of dose tables and of the "Technical Notes." These are intended primarily for the instructor and investigator, indicating the sources where more detailed information and different methods may be found.

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The image shows a close-up of a single page from an old, handwritten manuscript. The paper is a light cream or yellowish color, showing signs of age such as slight discoloration and some faint, irregular staining. There are two distinct lines of handwriting in a dark ink, likely iron gall. The script is a cursive hand, with letters that are somewhat compact and connected. The first line of text is positioned in the upper half of the page, and the second line is in the lower half. The handwriting is legible but has the characteristic fluidity and sometimes overlapping of letters found in historical cursive. The overall appearance is that of a well-preserved but clearly antique document.

PREFACE

THE following exercises are designed to introduce the student personally to some of the more important facts of pharmacology. They have been selected so as to present little difficulty to one versed in ordinary chemic and physiologic technic, and require but little help on the part of the instructor.

The pharmaceutic and toxicologic exercises (Part I) are confined strictly to the bare essentials needed by students who intend to become general practitioners of medicine. Especial stress has been laid on the facts which have a direct practical bearing.

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A LABORATORY GUIDE IN PHARMACOLOGY

INTRODUCTION

The Objects and Methods of Laboratory Instruction.—It seems quite superfluous at this time to insist on the great value of laboratory instruction. It may be well, however, to summarize the objects which it must keep in view. These consist in imparting information, in developing an understanding of the subject, and in acquiring a technical training. The information which can be derived directly from laboratory work forms the proper basis of didactic instruction: It facilitates the understanding of those facts which are deduced from experiments; it illustrates their value and their limitations; it impresses them on the memory. The training of a laboratory course cultivates manual dexterity and, what is more important, it fosters the "scientific spirit"—the judicial attitude of mind which requires the objective demonstration of statements and theories, and which deduces from these objective data the conclusions which they justify—no more and no less. The ultimate goal of this instruction should be to enable the student to deal critically and independently with the matter which is presented to him; to give him a more vital grasp of the whole subject of pharmacologic knowledge; and to generate and stimulate a healthy thirst for further information.

The course of instruction which will meet these requirements in the best attainable manner must vary somewhat with the resources at the command of the department; with the size of the classes; and with the special qualifications of the students and instructors. This applies particularly to the total time which can be devoted to laboratory work, and its apportionment to class demonstrations and to individual work by the students. The most thorough training would probably be obtained if the student were to perform every experiment for himself, with a minimum of aid from the instructor. The time which would be required for this purpose is, however, quite prohibitive; nor is this plan essential. Demonstrations—arranged in such a manner that every student can see the experiment, and so that as many as possible may assist in its performance—are almost as useful as regards the information acquired, and can be substituted for a considerable number of individual experiments in regard to the training; especially if the student has himself performed similar experiments. They cannot, however, replace individual work completely, and as much of this should be given as time and material permit. The demonstrations are advantageously shown in connection with the individual laboratory work; the students being called from their experiments to watch the results of the demonstrations. This economizes time when lengthy preparation or intermittent observations are involved; it facilitates the co-operation of the students and demonstrators; and it emphasizes the close relation of the demonstrations and of the individual work. Another expedient of economy,

which is extensively utilized in this course, consists in having parts of the class perform analogous experiments, but with different drugs; the results of each section being demonstrated and reported to the entire class. A great deal of time can also be saved by having the apparatus and reagents in good order, systematically arranged, and conveniently accessible. The student should co-operate in this by keeping his working-place orderly, neat, and clean.

Even with the closest management of the time it is naturally impossible to present every possible pharmacologic experiment to the class. Those experiments should be selected which demonstrate fundamental facts and methods in the simplest manner. Experiments which consume much time, or which are beset with special difficulties, or which are so exposed to accidents that they are more apt to fail than to succeed in the hands of elementary students, are not suited to the conditions of an ordinary laboratory course, and may be left to advanced students who wish to devote extra time to the subjects.

The mere performance of these experiments has only a very limited value if the student does not study them exhaustively. He should have a definite conception of the object of each experiment before he undertakes its performance; and he should render to himself an account of every step of the process, and of the conclusions to which it leads. The student's note-book is therefore a very essential part of the course. Nothing cultivates the powers of observation like the taking of careful, detailed notes during the progress of the experiment; while the critical faculty is stimulated by the condensation of these detailed results into brief and definite conclusions. This applies particularly to the animal experiments. The constancy or variability of the results are illustrated by comparing the results of different members of the class and of preceding classes. For this purpose it is well to appoint a class reporter for each exercise, with the duty of collecting and comparing all the results; these reports being kept on file for the use of succeeding classes. They should be read and discussed in the laboratory conferences.

Teachers differ in opinion as to whether the objects of the experiments and the expected results should be pointed out to the student in advance. In a pharmacology course the author believes that it is more useful to do so, on account of the complexity of the subject; and the large ground which has to be covered.

Relation of the Laboratory and Didactic Instruction.—The laboratory course may be treated either as an adjunct to, or as the basis of, the didactic instruction. If it is intended to illustrate the didactic teaching, it should keep step with the latter; the experiments should be arranged with reference to each drug. In the author's opinion, however, the course is much more valuable if it is made the basis of the pharmacologic instruction; if it is used to deduce the facts rather than to illustrate them. For this purpose the laboratory course should precede the didactic instruction; and the exercises should be arranged with a view to the pharmacology of particular organs, and the methods used in their investigation, rather than with regard to the individual drugs. If the conclusions are correctly drawn and summarized the student will enter on the didactic study with a fairly extensive, first-hand knowledge of the principal facts. The purpose of the didactic instruction will then be to correlate, apply, and extend these facts..

An elementary laboratory course is, of necessity, somewhat unevenly balanced. It is much better suited for the development of some facts than

of others; and undue stress seems therefore to be placed on the former. The "explanatory notes" and the "introductory discussions" are inserted to meet this objection. These are made as elementary as possible to keep them within the scope of the experimental knowledge of the student. Even with these, however, it is impossible at times to avoid an exaggeration of the laboratory side of the subject, and a comparative neglect of features which may be of greater practical therapeutic importance. This drawback should not be vital, for the didactic study should restore the balance. Attention should also be called to this subject by the demonstrators whenever necessary.

General Remarks on Note Taking.—The results of the experiments should be entered briefly in a special note-book. The method should be indicated sufficiently to make the notes understandable. Tracings should also be inserted when possible; either the original, or copies taken free hand, with tracing-paper, or blue prints. Unnecessary detail is to be avoided. The results should be followed by a brief statement of the conclusions which may be drawn from the experiment. These should only bear on principles, not on details. They should go no farther than the data of the experiment warrant. The "Questions" at the end of the Exercises may guide in this.

Students should always read the experiments before coming to the class. This is especially important when animals are to be used.

REFERENCE BOOKS

The following will be found useful in the laboratory, particularly for the details of methods:

- Abderhalden.—Handbuch der Biochemischen Arbeitsmethoden, Berlin.
- Association of Official Agricultural Chemists.—Methods of Analysis, United States Dept. Agric., Bur. Chem., No. 107.
- Autenrieth.—Detection of Poisons, translated by W. H. Warren.
- Edmunds and Cushny.—Laboratory Guide in Experimental Pharmacology, Ann Arbor, 1905.
- Fuehner.—Nachweiss und Bestimmung von Giften auf biologischen Wege, Berlin, 1911.
- Gadamer.—Lehrbuch der chemischen Toxicologie, Goettingen, 1909.
- Gooch.—Methods in Chemical Analysis, New York, 1912.
- Greene.—Experimental Pharmacology, Philadelphia, 1909.
- Harvard Apparatus Co.—Catalog.
- Hatcher and Sollmann.—Text-book of Materia Medica, Philadelphia, 1904.
- Heinz.—Handbuch der experimentellen Pathologie und Pharmakologie, Jena, 1905.
- Hoeber.—Physikalische Chemie der Zellen und Gewebe, Leipzig.
- Kobert.—Lehrbuch der Intoxicationen, Stuttgart, 1902.
- Korczynski.—Quantitative Bestimmung der Alkaloide, Berlin, 1913.
- Lenhartz.—Mikroskopie und Chemie am Krankenbett, Berlin, 1910.
- Merck.—Reagentien Verzeichniss, Berlin, 1913.
- National Formulary.
- Nelson.—Analysis of Drugs and Medicines, New York, 1910.
- Pharmacopœia of the United States.
- Pittenger.—Biochemic Drug Assay Methods, Philadelphia, 1914.
- Sahli.—Diagnostic Methods, Philadelphia, 1914.
- Sherman.—Organic Analysis, New York, 1912.
- Sollmann.—Manual of Pharmacology, Philadelphia.

Stewart.—Manual of Physiology, New York.

Sutton.—Volumetric Analysis, Philadelphia.

Tigerstedt.—Handbuch der Physiologischen Methodik, Leipzig.

Wester.—Darstellung phytochemischer Uebungs-präparate, Berlin, 1913.

SCHEDULE OF COURSES

The following detailed outline of the pharmacologic courses given in the author's laboratory may offer helpful suggestions:

COURSE I.—ELEMENTARY PHARMACY, GENERAL TOXICOLOGY, AND PRINCIPLES OF PRESCRIPTION WRITING

Two hours laboratory and one hour of didactic instruction per week in the first semester of the second year. Students work in pairs.

The numbers in the following refer to the weeks; (a) to the one-hour; (b) to the two-hour periods. "Optional" experiments are omitted.

(1a) Lecture and Demonstration: Pharmacognosy and Plant Constituents.

(1b) Laboratory: Assignment of Lockers. Reactions of Plant Constituents, Chapter I.

(2a) Lecture and Demonstration: Pharmaceutic Methods; Assaying. Recitation: On Lecture 1a.

(2b) Laboratory: Pharmaceutic Preparations, Chapter II, Exercise I to VIII.

(3a) Lecture: Liquid Pharmaceutic Preparations. Recitation: On Lecture 2a.

(3b) Laboratory: Pharmaceutic Preparations, Chapter II, Exercise VIII to XIV.

(4a) Lecture: Solid Pharmaceutic Preparations; Solubilities. Recitation: On Lecture 3a.

(4b) Lecture: Incompatibilities. Laboratory: Incompatibilities, Chapter III, Exercise I to III.

(5a) Lecture and Demonstration: Metrology. Recitation: On Lecture 4a.

(5b) Recitation: On Lecture 5a. Laboratory: Incompatibilities, Chapter III, Exercise IV to VI.

(6a) Recitation: Incompatibilities. Review: Metrology.

(6b) Lecture and Demonstration: Toxicologic Analysis and Assaying, Chapter IV.

(7a) Recitation: On Laboratory 6b. Review: Incompatibilities and Solubilities.

(7b) Laboratory: Tests for Important Drugs, Chapter V to VIII.

(8a) Written Test on Text and Laboratory "Questions." Assignment of experiments and reporters for Exercise XIII.

(8b) Laboratory: Flavors, Chapter XIII (part). Excretion of Drugs, Chapter XV (part).

(9a) Lecture: Treatment of Poisoning. Prescription Writing.

(9b) Conference: On Laboratory 8b. Laboratory: Flavors, Chapter XIII (part). Excretion of Drugs, Chapter XV (part).

- (10a) Lecture: Flavors and Colors.
Recitation: On Lecture 9a.
- (10b) Conference: On Laboratory 9b.
Laboratory: Colors, Chapter XIV.
Excretion of Drugs, Chapter XV (part).
Prescription Practice (in sections, alternating with laboratory work).
Study Materia Medica Lessons 1 and 2.
- (11a) Recitation: On Lecture 10a and Materia Medica Lessons 1 and 2.
Conference: On Laboratory 10b.
- (11b) Laboratory: Excretion of Drugs, Chapter XV (finish).
Chemic Antidotes: Chapter XVI.
Prescription Practice.
Study Materia Medica Lessons 3 and 4.
- (12a) Lecture: Treatment of Disease; Chemical and Physical Basis of Pharmacology.
Recitation: Materia Medica Lessons 3 and 4.
Conference: Laboratory 11b.
- (12b) Laboratory: Absorption and Selective Solvents, Chapters XVII and XVIII.
Prescription writing Practice.
- (13a) Lecture: Manifestations of Pharmacologic Action.
Recitation: On Lecture 12a.
- (13b) Conference: On Laboratory 12b.
Laboratory: Osmosis, etc., Chapter XIX to XXI.
Prescription Practice.
- (14a) Lecture: Administration of Drugs.
Recitation: On Lecture 13a.
- (14b) Conference: On Laboratory 13b.
Laboratory: Hemolysis, Irritants, etc., Chapter XXII to XXVII.
Prescription Practice.
- (15a) Lecture: Conditions Influencing Drug Actions.
Recitation: On Lecture 14a.
- (15b) Conference: On Laboratory 14b.
Laboratory: Antiseptics, Ferments, etc., Chapter XXVIII to XXXI.
Prescription Practice.
- (16a) Recitation: On Lecture 15a.
Conference: On Laboratory 15b.
- (16b) Written Test on Text, Prescription Writing and Laboratory "Questions," Identification of Specimens.
Assignment of Lockers for Animal Work.

COURSE II.—EXPERIMENTAL PHARMACODYNAMICS

Three hours of laboratory work and two hours of conferences per week in the second semester of the second year. Students work in groups of six, divided into subgroups A and B. The experiments are arranged for five full groups.

The syllabus is shown in the following table, which also gives the group and number (A to F) of the students who act as *class reporters* for each experiment. It is their duty to collect the individual reports and present the significant results at the "conferences."

First Day.—Chapter XXII.—Localization of Actions; Stimulants and Depressants

Demonstrations:

Exercise I.—Location and Type of Convulsions (Frogs):

(Reporter I, A)

Experiments: 1. Strychnin Convulsions.

3. Nature of Stimulus.

7. Cerebral Lobes on Convulsions.

8. Picrotoxin.

10. Veratrin.

11. Caffein Convulsions.

12. Caffein Rigor.

Exercise II, 8.—Central Depressants:

(Reporter II, D)

Experiment 8. Anesthesia of "Salted Frog."

Exercise IV.—Curare Actions:

(Reporter IV, D)

Experiments: 1. Symptoms of Curare.

2. Location.

3. Bernard Experiment.

8. Systemic Nicotin.

9. Nicotin in Smoke.

Exercise V.—Local Anesthesia:

(Reporter V, D)

Experiments: 1. Anesthesia of Cornea.

9. Freezing of Nerve.

Class Work.

A Groups:

Exercise I, 5.—Location of Experiment I, 5.

Strychnin Tetanus.

(Reporter I, A)

Exercise III.—Reflex Time.

(Reporter III, A)

Exercise V.—Local Anesthesia.

(Reporter V, D)

Experiment 1. De-

mulcents.

Experiment 3. Anes-

thesia of Tongue.

Experiment 5. Co-

cain, HCN.

II

Exercise I, 5.

III

Exercise I, 5.

IV

Exercise I, 5.

V

Exercise I, 5.

5. Strychnin.

Exercise V, 3.

6. Epinephrin-

cocain.

6. Morphin.

7. Apomorphin.

Exercise V, 3.

4. Cocain-alypin.

5. Ether.

4. Chloral.

7. Saponin.

5. Physostigmin.

Exercise V, 3.

4. Cocain-quinin-

urea.

B Groups:

Exercise II.—Central Depress-

ants (Frogs).

(Reporter II, D)

Exercise IV.—Curare Action.

(Reporter IV, D)

Exercise V.—Local Anesthesia.

(Reporter V, D)

4. Curare.

5. Physostigmin.

3. Anesthesia of

Tongue.

4. Cocain-novocain.

3. Alcohol.

4. Nicotin.

5. Physostigmin.

Exercise V, 3.

4. Cocain-stovain.

4. Cocain-tropo-

cain.

Second Day for A Groups; Third Day for B Groups.—Chapter XX XIII.—Muscular Contraction

Demonstrations:

- (Reporter I, A) Exercise III.—Maximal Load.
 (Reporter I, A) Exercise IV.—Fatigue.
 (Reporter III, B) Exercise VI.—Osmotic Effects on Muscle and Nerve.
 (Reporter V, B) Exercise VIII.—Salt on Vitality of Heart.
 (Reporter V, B) Exercise IX, 1.—Ciliary Paralysis.
 (Reporter V, B) Exercise IX, 2.—Germination of Seeds.
 (Reporter I, F) Exercise X.—Astringents.

<i>Class Work:</i>					
Exercise I.—Form of Contraction.	I Groups: Caffein.	II Theobromin.	III Quinin.	IV Potassium.	V Alcohol.
(Reporter I, B)					
Exercise II.—Veratrin Effect.	Fatigue.	Temperature.	Potassium.	Ether.	Secondary contraction.
(Reporter II, B)					
Exercise VII.—Rhythmic Contraction.	1. Citrate and Ca.	2. Citrate and Ba.	3. Ba. and Ca.	4. Citrate and K.	5. Fluorid and Ca.
(Reporter IV, B)					

Third Day for A Groups; Second Day for B Groups.—Chapter XX XIV.—Smooth Muscle; Peristalsis

Demonstrations:

- Exercise I.—Peristalsis of Exposed Intestine: (1) Bayliss-Starling Reflex. (2) Local Irritation. (3) Physostigmin. (4) Barium. (5) Atropin.
 (6) Nicotin on Ganglia. (7) Pilocarpin. (8) Pfituitrin. (9) Atropin. (10) Barium.
 (Reporter II, F)

<i>Class Work:</i>					
	I	II	III	IV	V
Exercise VI.—Autonomic Poisons on Intestine.	Epinephrin.	Pituitary-atropin.	Pilocarpin-atropin.	Nicotin-atropin.	Barium-atropin.
(Reporter III, F)					
Exercise VII.—Salt Actions, Intestine.	Sod. Sulph.	Sod. Citrate.	Magnes. Chlor.	Calcium Chlor.	2 per cent. NaCl.
(Reporter IV, F)					
Exercise VIII.—Autonomic Poisons on Uterus.	Epinephrin.	Pituitary.	Quinin.	Ergot.	Hydrastis.
(Reporter V, F)					
Exercise IX.—Arterial Rings.	Epinephrin-nitrite.	Nitrite-epinephrin.	Barium-nitrite.	Digitalis-nitrite.	Physostigmin-nitrite.
(Reporter V, F)					

Sixth Day.—Chapter XXVII.—Pupils, Glands, Bronchioles, Anaphylaxis

Demonstrations:

- Exercise I.—Localization of Atropin Mydriasis.
(Reporter V, B)
- Exercise V.—Pilocarpin and Atropin in Living Animals.
(Reporter II, A)
- Exercise VII.—Bronchial Tone in Intact Animals.
(Reporter II, A)
- Exercise VIII.—Treatment of Bronchial Spasm, Perfused Lung.
(Reporter II, A)
- Exercise X.—Anaphylaxis, Guinea-pig; Atropin.
(Reporter IV, A)
- Exercise XII.—Calcium in Dionin Chemosis.
(Reporter IV, A)
- Class Work.

A Groups:

- | | | | | |
|--|---------------------------------------|---------------------------------------|---------------------------------------|--------------------------------------|
| Class work. | | | | |
| <i>A Groups:</i> | | I | II | III |
| Exercise II.—Local Application | 1. Atropin; Pilocarpin; Physostigmin. | 2. Physostigmin. | 3. Pilocarpin. | 4. Cocain. |
| Mammalian Eye. | | | | 5. Dionin. |
| (Reporter V, B) | | | | |
| <i>B Groups:</i> | | | | |
| Exercise III.—Frog Pupil. | 1. Pilocarpin; Physostigmin. | 2. Atropin; Pilocarpin; Physostigmin. | 3. Nicotin; Pilocarpin; Physostigmin. | 4. Cocain; Pilocarpin; Physostigmin. |
| (Reporter V, B) | | | | |
| Exercise VI.—Reflex Secretion of Saliva. | Exercise VI. | Exercise VI. | Exercise VI. | Exercise VI. |
| (Reporter II, A) | | | | |
| <i>B Groups:</i> | | | | |
| Exercise III.—Frog Pupil. | 1. Pilocarpin; Physostigmin. | 2. Atropin; Pilocarpin; Physostigmin. | 3. Nicotin; Pilocarpin; Physostigmin. | 4. Cocain; Pilocarpin; Physostigmin. |
| (Reporter V, B) | | | | |
| Exercise VI.—Reflex Secretion of Saliva. | Exercise VI. | Exercise VI. | Exercise VI. | Exercise VI. |
| (Reporter II, A) | | | | |
| | | | | 5. Epinephrin. |

Seventh Day.—Chapter XXVIII.—Absorption, Excretion, etc.; Emetics, etc.

Demonstrations:

- Exercise I.—Rapidity of Absorption by Various Channels—Epinephrin and Strychnin. (Reporter V, D)
Exercise II.—Rapid Absorption in Oral Administration—Nicotin and Hydrocyanic Acid. (Reporter V, D)
Exercise III.—Rapid Absorption of Gases by Lungs—CO Poisoning. (Reporter V, D)
Exercise VII.—Pulmonary Excretion—Hydrogen Sulphid. (Reporter I, A)
Exercise VIII.—Interaction of Iodid and Calomel; Morphin; Distribution of Fluorescein. (Reporter I, A)

<i>Class Work:</i>							
Whole Groups: (Reporter I, A)	A Groups: (Reporter II, A)	Groups: I		II	III	IV	V
		Exercise IV.—Absorption, Strych. Oral and Hypod.		Exercise V.—Absorption, Chloral, Oral and Rectal.	Exercise VI.—Colloid on Absorption of Strych.	Exercise XI.—Atropin, Dog and Rabbit.	Exercise XIV.—Apomorphin on Different Animals.
Exercise XVI.—Emetics, Copper Sulphate.		Exercise XVI.—Zinc Sulphate.	XVI.—Tartar Emetic.				
(1)		(2)					
XVII.—Morphin and Apomorphin.		XVII.—Morphin and Zinc Sulphate.	XVIII.—Bismuth and Zinc Sulphate.				
B Groups: (Reporter II, A)							

Eighth Day.—Chapter XXXIX.—Temperature; Central Depressants; Reflex Irritants

Demonstrations:

- Exercise XI.—Acid Intoxication.
(Reporter V, A)
- Exercise XIII, 4.—Mouse-test for Morphint.
(Reporter IV, A)
- Exercise XV.—Magnesium and Calcium, Rabbit.
(Reporter IV, A)
- Exercise XX.—Uranium Hydrops.
(Reporter V, A)
- Exercise XXIII.—Irritants on Blood-pressure and Respiration.
(Reporter V, A)
- Exercise XXIV.—Arsenic on Circulation.
(Reporter V, A)
- Class Work.

<i>A Groups:</i>						
Temperature: (Reporter III, A) Central Depressants. (Reporter IV, A) Irritants. (Reporter V, A)	I and XVII, 1.—Chloral, Cat.	II, VIII, XIII, 3.—Morphin, Rabbit.	III.—Santonin,	IV.—Cocain.	V	
	XVII, 3.—Chloral Caffein.	XIII, 1.—Morphin, Dog.	XVI, 1.—Alcohol, Control.	XVI, 3.—Alcohol-caffein Antagonism.		
	XVIII, 1.—Colchicum.				
	XVIII, 3.—Arsenic.				

V

Exercise XIV.—Apomorphin on Different Animals.

V

VI, 1.—Albumose.
VI, 2.—Antipyrin.

B Groups:

Temperature.

(Reporter III, A)

Central Depressants.

(Reporter IV, A)

Irritants.

(Reporter V, A)

(Reporter III, D)

Class Work.

A Groups:

I	II	III	IV	V
.....	V.—Beta-tetrahydro-naphthylamin.	VI, 3.—Albumose and Antipyrin.
XVII, 2.—Chloral and Heart.	XIII, 2.—Morphin, Cat.	XIV, 1.—Cannabis.		
XVII, 4.—Chloral and Strychnin.	XVI, 2.—Alcohol and Emesis,	XVI, 4.—Alcohol-caffein Synergism.	
.....	XVIII, 2.—Mercuric Chlorid.			

Ninth Day.—Chapter XL.—Consultants; Treatment of Poisoning

I	II	III	IV	V
III.—Camphor Convulsions.	I.—Strychnin podermic.	X.—Strychnin and Chloral.	VIII.—Strychnin and Lavage.	VII.—Strychnin and Charcoal.
IV.—Camphor and Bromid.	IX.—Strychnin and Artificial Respiration.	II.—Strychnin—Stomach.	VI, 1.—Strychnin and Permanganate.	VI, 2.—HCN and Permanganate.

*B Groups:**Demonstrations:*

Exercise I.—Volume of Air: 1. Morphin on Normal Rabbit; 2. Morphin in Hyperpnea; 3. Toxic Dose of Morphin; 4. Camphor; 5. Caffein. (Reporter I, D)

Class Work:

Group I

Exercise IV.—Respiration of Normal Rabbit:

(Reporter II, D)

1. Auditory Reflex.

2. Chloral.

3. Hypodermic Water.

4. Caffein, Hypodermic.

II

Exercise V.—Respiration of Normal Rabbit:

(Reporter II, D)

1. Hypodermic Water.

2. Hypodermic Alcohol.

3. Strychnin, Therap.

4. Atropin, Therap.

III

Exercise VI.—Respiration of Normal Rabbit:

(Reporter II, D)

1. Ammonia Reflex.

2. Morphin, Therap.

3. Morphin, Toxic.

4. Nicotin.

IV

Exercise VII.—Respiration and Blood-pressure Tracing:

(Reporter IV, D)

1. Lactic Acid.

2. Caffein.

3. Camphor.

4. Anal Sphincter.

5. Sciatic Stimulation.

6. Strychnin, Therap.

7. Strychnin, Toxic.

V

Exercise VIII.—Respiratory and Blood-pressure Tracing:

(Reporter IV, D)

1. Ammonia Inhalation.

2. Ammonium Chlorid.

3. Mild Asphyxia.

4. Severe Asphyxia.

5. Apnea.

6. Strychnin, Therap.

7. Strychnin, Toxic.

Tenth Day.—Chapter XLI.—Respiration

Glass Work: Group I

12. Reflexes.
13. Insufficient Aëration.
14. Intravenous Anesthesia.
15. Chloroform Poisoning.
16. Resuscitation (see Exercise VII, last column).

II

12. Cardiac Failure by Excessive Epinephrin.

III

12. Cardiac Failure by Excessive Epinephrin.
- Twelfth Day.—Chapter XLIII.—Vasomotor Drugs; Treatment of Circulatory Collapse*
Class Work
 (Reporters: B members of each Group)

*Group and Exercise:**I*

Nitrite and Epinephrin;
 Relation of Effect to
 Blood-pressure; Stephen-
 Hale Manometer; Trac-
 ings of Blood-pressure
 and Respiration.

1. Epinephrin } Hale.
2. Amyl Nitrite }
3. Splanchnic Stimulation.
4. Nitroglycerin.
5. Splanchnic Stimulation during Nitroglyc.
6. Compression of Aorta.
7. Nitroglyc. during Com-
pression of Aorta.
8. Compression during Nitroglyc. Fall.
9. Epinephrin.
10. Epinephrin during Ni-
troglyc.
11. Nitroglyc. during Epi-
nephrin.
12. Nitroglyc. during Hem-
orrhage.
13. Strophanthus.
14. Nitroglyc. during Stro-
phanthus.

II

Peripheral Vasomotor
 Drugs on Blood-pressure
 and Kidney Volume; In-
 spection of Intestinal Ves-
 sels.

1. Strychnin, Therap. Dose.
2. Amyl Nitrite.
3. Epinephrin.
4. Pituitary.
5. Ergot.
6. Tyramin.
7. Histamin.
8. Cholin.
9. Cotarnin.
10. Hydrastis.
11. Hydrastinin.
12. Nicotin.

III

Peripheral and Central
 Vasomotor Drugs on
 Blood-pressure, Intesti-
 nal Volume and Respira-
 tion. Treatment of Pep-
 tone Shock.

1. Strychnin, Therap. Dose.
2. Sodium Nitrite.
3. Epinephrin.
4. Alcohol.
5. Veronal.
6. Peptone Shock.
7. Treatment of Shock.
8. Saline Infusion.
9. Epinephrin.
10. Strophanthus.
11. Epinephrin during
Stroph.

IV

Peripheral and Central
 Vasomotor Drugs on
 Blood-pressure and Car-
 dioplethysmogram.

1. Strychnin, Therap. Dose.
2. Nitroglycerin.
3. Hemorrhage.
4. Epinephrin.
5. Aortic Compression.
6. Pituitary.
7. Phenol.
8. Chloral.
9. Strophanthus.
10. Arsenic.

V

Reactions of Vasomotor
 Center (Perfusion Meth-
 od).

1. Asphyxia.
2. Hemorrhage.
3. Reinjection of Blood.
4. Asphyxia.
5. Nitroglycerin.
6. Epinephrin.
7. Strychnin, Ther. Dose.
8. Chloroform Inhalation.
9. Caffein.
10. Cevadin.
11. Atropin.
12. Strophanthus, Therap.
13. Strophanthus, Toxic.

Thirteenth Day.—Chapter XLIV.—Change in Heart-rate, etc.
(Reporters: A members of each group)

Group and Exercise:

I		II		III		IV		V	
Heart-rate on Blood-pressure and Cardiac Excursions (Cardiomyograph).		On Blood-pressure and Cardiac Excursions (Cardiomyograph).		On Blood-pressure and Organ Volume (Kidney Oncometer).		On Blood-pressure and Urine Flow.		Heart-rate and Respiration as Influenced by Blood-pressure.	
1. Weak Vagus Stimulation.		1. Weak Vagus Stimulation.		1. Weak Vagus Stimulation.		1. Weak Vagus Stimulation.		1. Nitroglycerin.	
2. Maximal V. St.		2. Maximal V. St.		2. Maximal V. St.		2. Maximal V. St.		2. Hemorrhage.	
3. Cevadin.		3. Cevadin.		3. Cevadin.		3. Cevadin.		3. Nitroglycerin and Compression of Aorta.	
4. Section of Vagi.		4. Spartein.		4. Dog's Urine.		4. Atropin.		4. Epinephrin.	
5. Cevadin after Section of Vagi.		5. Pilocarpin.		5. Ouabain.		5. Barium.		5. Compression of Aorta.	
6. Strophanthus.		6. Digitalis.			6. Strophanthus and Hemorrhage.	

Fourteenth Day.—Chapter XLV.—Myocardial Depressants and Tonics
(Reporters: F members of group each)

Group and Exercise:

I		II		III		IV		V	
Cardiac Depressants on Blood-pressure and Oncometer.		Circulatory Drugs on Blood-pressure and Vein Pressure.		Cardiac Stimulants and Depressants on Cardiomyogram.		Circulatory Drugs on Pressure in Pulmonary Artery.		Cardiac Drugs on Cardioplethysmogram.	
1. Aconite, Therap. Dose.		1. Weak Vagus Stimulation.		1. Caffein, Therap. Dose.		1. Weak Vagus Stimulation.		1. Asphyxia and Recovery.	
2. Antipyrin.		2. Maximal Vagus Stimulation.		2. Chloroform.		2. Maximal Vagus Stimulation.		2. Strychnin, Therap. Dose.	
3. Phenol.		3. Nitroglycerin.		3. Spartein.		3. Nitroglycerin.		3. Potassium.	
4. Veratrum.		4. Epinephrin.		4. Digitalis.		4. Epinephrin.		4. Camphor.	
5. Aconite, Toxic Dose.		5. Ergot.		5. Caffein Rigor.		5. Ergot.		5. Veratrum.	
6. Chloroform Rigor.		6. Barium.			6. Strophanthus.		6. Strophanthus.	

Group and Exercise:

Urine Flow (Drop Recorder); Tracings of Blood-pressure and Respiration.

1. Sulphate Diuresis.
2. Epinephrin.
3. Spartein.

Urine Flow; Blood-pressure Tracing.

1. Osmotic Absorption.
2. Chlorid Diuresis.
3. Strong Vagus Stimulation.
4. Theobromin.
5. Hemorrhage.
6. Saline Infusion.
7. Injection of Blood.

Urine Flow and Kidney volume. Blood-pressure Tracing.

1. Osmotic Absorption.
2. Hypertonic Diuresis.
3. Strong Vagus Stimulation.
4. Theophyllin.

Urine Flow and Kidney
volume. Blood-pressure
Tracing.

1. Glucose Diuresis.
2. Amyl Nitrite.
3. Caffein.
4. Hemorrhage.
5. Saline Infusion.
6. Reinnecction of Blood.

Urine Flow and Kidney
volume. Blood-pressure
Tracing.

1. Osmotic Absorption.
2. Locke Diuresis.
3. Epinephrin.
4. Pituitary.

Aortic Stenosis—Cardio-plethysmograph, Blood-pressure.

4. Aortic Stenosis.
5. Weak Vagus Stim.
6. Strong Vagus Stim.
7. Saline Infusion.
8. Strophanthus.

Hydropericardium—Blood-pressure and Inspection.

8. Pericardial Pressure.
9. Weak Vagus Stim.
10. Strong Vagus Stim.
11. Saline Infusion.
12. Strophanthus.

Myocarditis—Blood-pressure and Inspection.

5. Alcohol Injection.
6. Weak Vagus Stim.
7. Strong Vagus Stim.
8. Saline Infusion.
9. Strophanthus.

Aortic Aneurysm—Blood-pressure.

7. Aortic Aneurysm.
8. Weak Vagus Stim.
9. Strong Vagus Stim.
10. Saline Infusion.
11. Strophanthus.

Coronary Sclerosis—Cardiomyogram, Blood-pressure.

5. Lycopodium Injection.
6. Weak Vagus Stim.
7. Strong Vagus Stim.
8. Amyl Nitrite.
9. Strophanthus.

COURSE III.—SYSTEMATIC PHARMACOLOGY: DRUGS WITH PREDOMINANT LOCAL ACTION

One hour, lecture or recitation, per week in the fourth semisemester of the second year.

1. Ferments and Nutrients.
2. Emollients and Demulcents.
3. General Phenomena of Irritation, Corrosion and Astringents.
4. Inorganic Irritants and Astringents.
5. Irritant Volatile Oils.
6. Irritant Volatile Oils and Physical Irritants.
7. Stomachics, Cathartics.
8. Cathartics, Anthelmintics.

COURSE IV.—SYSTEMATIC PHARMACOLOGY**DRUGS WITH PREDOMINANT SYSTEMIC ACTION**

Four hours per week, didactic, in the first semisemester; and three hours per week in the second semisemester of the third year. (L = Lectures; R = Recitations.)

L 1. Strychnin.

L 2. Caffein.

R 1. On L 1 and 2.

L 3. Morphin.

L 4. Morphin, hydrastis.

L 5. Cannabis, cocain.

R 2. On L 3, 4, and 5.

L 6. Autonomic drugs.

L 7. Autonomic drugs.

L 8. Atropin, scopolamin.

R 3. On L 6 and 7.

L 9. Pilocarpin to curare.

L 10. Epinephrin, pituitary, thyroid.

L 11. Ergot to nitrites.

R 4. On L 8 and 9.

L 12. Digitalis.

L 13. Digitalis.

L 14. Camphor to colchicum.

R 5. On L 10 and 11.

L 15. Apomorphin to heat regulation.

L 16. Antipyretics.

L 17. Benzol antiseptics.

R 6. On L 12 and 13.

L 18. Benzol antiseptics.

L 19. Miscellaneous antiseptics, sera, vaccines.

L 20. Narcosis theories, alcohol.

R 7. On L 14, 15, and 16.

L 21. Alcohol.

- L 22. Anesthetics.
- L 23. Anesthetics.
- R 8. On L 17, 18, and 19.
Written tests and specimens, Lectures 1 to 19 inclusive.
- L 24. Hypnotics, gases, cyanids.
- R 9. On L 20 and 21.
- L 25. Physics of salt action.
- L 26. Physics of salt action.
- R 10. On L 22, 23, and 24.
- L 27. Physiology of salt action.
- L 28. Cathartic salts, water, diuresis.
- R 11. On L 25 and 26.
- L 29. Cathions.
- L 30. Anions.
- R 12. On L 27 and 28.
- L 31. Reaction.
- L 32. Metals, inorganic compounds.
- R 13. On L 29, 30, and 31.
- L 33. Organic arsenic compounds.
- L 34. Antimony, bismuth, iron.
- R 14. On L 32 and 33.
- L 35. Radium, silver.
- L 36. Mercury.
- R 15. On L 34 and 35.
- L 37. Lead, phosphorus.
- R 16. On L 36 and 37.
Written tests and specimens on Lectures 20 to 37.
Examination on entire subject.

PART I

CHEMIC EXERCISES

Introductory Remarks.—Before beginning on the laboratory work the student should check the contents of his locker and familiarize himself with the reagents on the shelves (see Appendix). These are arranged alphabetically. Remember that they are to be replaced in their proper position as soon as used. The student should supply himself with towel, soap, matches, scratch-pad, and dissecting instruments. He should keep his working-place clean and neat.

The experiments, explanatory remarks, and references should be assigned and read before coming to the class. Cross-references to other experiments (*e. g.*, "Consult Exercise so and so") mean that these experiments are to be read, but not to be performed, at this time. The student should reflect on the object and conclusions of the experiment while it is in progress. He should take account of all the experiments performed in the course, including those shown as demonstrations or assigned to other members of the class. Two students may collaborate in the chemic experiments.

Successful results should be checked in the book and the questions answered in the note-book.

If an experiment is unsuccessful, it should be repeated. In the event of a second failure, the student should call on the demonstrator for help. *Every unusual or atypical result should be reported.*

Additional apparatus is furnished on written requisition. The special material needed for each experiment is noted at the bottom of each page (*S. M.*).

CHAPTER I

GENERAL REACTIONS OF PLANT CONSTITUENTS

(It is assumed that the student is familiar with the characters of glucose, cane-sugar, starch, proteins, and fats. Should this not be the case, they should be studied before the following experiments are made.) Two students may work together.

EXERCISE I.—ALKALOIDS¹

1. **Alkalinity.**—Place a drop of 1 per cent. nicotin on red litmus paper: blue color.

2. **Precipitation Reactions.**—Place on slides a few drops of 1 : 1000 acidulated quinin sulphate solution, mix with a drop of the following, and note the amorphous precipitates:

- | | |
|--|------------|
| (a) Iodin in KI | = Reddish. |
| (b) Mercuric Potassium Iodid
(Mayer's Reagent.) | = White. |
| (c) Picric Acid | = Yellow. |
| (d) Tannin (about 1 per cent.) | = Gray. |
| (e) Phosphotungstic Acid ² | = White. |

S. M.—Nicotin, 1 per cent.

¹ Similar reactions are given by other organic bases, *e. g.*, pyridin and quininol.

² *Phosphotungstic Acid*: A 10 per cent. solution in 4 per cent. HCl.

3. **Solubility Characters of Alkaloids and their Salts.**—In a test-tube make about 5 c.c. of an acidulated 1 : 1000 solution of quinin sulphate distinctly alkaline by NaOH solution: a precipitate of free alkaloid is thrown down (free alkaloids are generally insoluble in water, while their salts are soluble). Add about 10 c.c. of ether and shake with a gentle rotatory motion. Draw off the ethereal solution from the top with a pipet, and again shake the watery solution with 5 c.c. of ether. Again draw off the ether. Acidulate some of the remaining watery solution and test it with mercuric potassic iodid, observing that there is no or very little precipitate (the free alkaloid being completely extracted by the ether). Shake the ethereal solution with some dilute sulphuric acid. Draw off a little of the acid solution from the bottom, and test with mercuric potassic iodid: a precipitate occurs.¹ (The acid converted the quinin into the sulphate, which is soluble in water and insoluble in ether.)

4. (Optional) **Lassaigne's Test for Nitrogen.**—Place a knife-pointful of dry quinin sulphate in a dry test-tube. Take a piece of metallic Na, size of small pea, dry with blotting-paper, and add to quinin. Heat red hot and plunge into beaker with a little water. Filter. Add a few drops FeSO_4 . Let stand five minutes. Acidulate with conc. HCl and heat: Greenish or blue color or precipitate of prussian blue.

Note the peculiar odor of quinolin, a decomposition product of quinin.

Explanatory Notes.—On heating with sodium, the N of quinin (and other nitrogenous substances) gives sodium cyanid; treated with a ferrous salt, this gives the ferrocyanid; on adding acid, this forms ferricyanid with the ferric salt formed from the ferrous sulphate.

5. (Optional) **Microchemic Reactions.**²—Alkaloidal precipitates often present a crystalline character, which may be useful in their identification. This is illustrated by the following examples. (Mix the solutions on a slide, and examine from time to time with low-power microscope, until typical crystals are seen.)

(a) 5 drops of 2 per cent. morphin sulphate and 1 drop 10 per cent. NH_4OH : rapid formation of needles. (Rub with a glass rod if necessary.)

(b) 5 drops of $\frac{1}{10}$ per cent. nicotin and excess of picric acid: at first a fine precipitate; later stellate crystals.

(c) Substitute 1 per cent. atropin sulphate for nicotin in (b): feathery crystals and stellate groups.

(d) 1 per cent. strychnin sulphate and potassic bichromate solutions: fine rosettes of needles at once.

6. (Optional) **Preparation of Alkaloids.**—Directions are given in D. H. Wester, "Anleitung zur Darstellung phytochemischer Uebungspraeparate," Berlin, 1913. The preparation of caffen and piperin are convenient examples. The preparation of individual alkaloids is also described in Abderhalden's Handb., 2, 904.

QUESTIONS

(a) State the principal properties of alkaloids (reaction, precipitants, solubility, characteristic element).

(b) How would you test a solution for the presence of alkaloids?

(c) Why is it necessary to apply several tests?

(d) How would you extract an alkaloid from a solution of its salts?

(e) Should alkaloids be prescribed with iodin or tannin?

TECHNICAL REFERENCES

Qualitative and quantitative tests, Abderhalden's Handb., 6, 118; Gadamer, Lehrb. d. chem. Toxicologie.

¹ Water saturated with ether and acid may give a precipitate with Mayer's reagent, even in the absence of alkaloids; but this non-alkaloidal precipitate dissolves on adding an equal volume of water (A. H. Clark; reference, Amer. Jour. Pharmacy, 1900, 176).

² T. G. Wormley, "Microchemistry of Poisons," Philadelphia, 1885.

EXERCISE II.—GLUCOSIDS

1. Test a little fresh 1 per cent. solution of salicin (a glucosid) for reducing sugar by Trommer's test¹: negative.

2. **Decomposition by Acids.**—To another portion of the solution add $\frac{1}{10}$ volume of 10 per cent. sulphuric acid; boil in water-bath for ten minutes; make alkaline with NaOH and apply Trommer's: positive.

3. **Decomposition by Ferments.**—To another portion of the solution add some saliva and heat in water bath at 40° C. for half-hour; test for sugar: positive.

4. Note difference in sweetness of alkaline and acidulated fluidextract of licorice. (The sweet glucosid, glycyrrhizin, like many glucosids, is a feeble acid, held in solution by ammonia and precipitated by strong acids.)

5. (Optional) **Brunner-Pettenkofer's Reaction** (Given by Glucosids and Sugars).—Dissolve some glucosid and purified ox-bile in water, and pour carefully on a layer of concentrated sulphuric acid: red ring at contact; on agitation the whole fluid is colored red.

6. (Optional) **Decomposition by Emulsin.**—Preparation and tests of emulsin, Abderhalden's Handb., 3, 391; 7, 760.

QUESTIONS

- What is the characteristic property of glucosids?
- How do they differ from ordinary carbohydrates?
- Why is it inadvisable to prescribe solutions of glucosids with acids?
- How would glucosids be affected in the alimentary canal?
- How would you distinguish between a glucosid and an alkaloid?
- How would you separate an alkaloid and a glucosid from a solution containing both?

TECHNICAL REFERENCES

Preparation, Reactions, and Synthesis of Glucosids, Abderhalden's Handb., 7, 732.

EXERCISE III.—SAPONINS

Saponins give the typical reactions of glucosids. They lake blood corpuscles (see Chapter XXII).

1. **Foaming.**—Shake a few drops of a tincture of soap-bark (which is rich in saponin) with a little water: considerable foam is produced, which subsides slowly.

2. **Emulsification.**—Add 25 drops of the soap-bark tincture to about an inch of cotton-seed oil. Shake. Add an inch of water and shake: a smooth mixture (emulsion) is formed. Add alcohol: the emulsion persists.

3. (Optional) **Color Reaction.**—Concentrated sulphuric acid dissolves saponins with a yellow to brick-red color, passing gradually through red to violet.

QUESTIONS

- How would you detect saponin in a plant extract?
- What is the explanation of the saponin action?
- What practical uses can be made of these actions?

S. M.—Salicin, 1 per cent.; fluidextract licorice, plain and acidulated.

¹ *Trommer's Test.*—Make strongly alkaline with NaOH and add dilute cupric sulphate, drop by drop, until a slight permanent precipitate of cupric hydrate appears. Boil: glucose causes yellow or brownish-red precipitate of cuprous oxid.

TECHNICAL REFERENCES

Preparation, etc., Abderhalden's Handb., 2, 970; *Detection in frothing liquids*, etc., Gadamer, 446; Loncheux, ref., Yearbook Amer. Pharm. Assoc., 1, 448, 1912; *Determination*, Korsakoff, 1912, ref., Chem. Abstr., 7, 803; *Bio-estimation in drugs*, Kobert, 1912, ref., Yearbook Amer. Pharm. Assoc., 1, 446.

EXERCISE IV.—CATHARTIC EMODIN PRINCIPLES

1. **Borntraeeger's Reaction for Emodin or Chrysophanic Acid.**—To an infusion of rhubarb add a few drops of ammonia: red color.

2. (Optional) **Hirschsohn's Reaction for Aloins.**—Mix 10 c.c. of 1 : 1000 aloin solution with 1 drop of 10 per cent. copper sulphate and of 2 per cent. hydrogen peroxid; boil: red color (hindered by alcohol, acids, and alkalies).

3. (Optional) **Stacy's Reaction for Aloes.**—A delicate reaction with ferricyanid, the tints distinguishing the varieties (Ref., Amer. Jour. Pharm., 88, 262, 1916).

QUESTIONS

(a) How would you determine whether a patient is taking an emodin cathartic? (The chrysophanic acid passes into the urine; however, santonin and phenolphthalein urines give similar reactions.)

(b) What change would occur in a rhubarb urine on standing?

TECHNICAL REFERENCES

Assay of Emodin Drugs, E'we and Vanderkleed, 1913, Jour. Amer. Pharm. Assoc., 2, 979; Gadamer, 422; Daels, 1913, ref., Jahrb. Pharmacie, 73, 6; *Rhubarb*, colorimetric determination of value, Tsirch, 1904, Jahrb. Pharm., 111; *Detection of Emodin drugs in presence of Phenolphthalein*, L. E. Warren, 1914, Amer. Jour. Pharm., 86, 444; *Determination of Drastic Purgatives*, Gadamer, 426; *Colocynthin*, Test for, Venturoli and Vervi, 1909, ref., Jahrb. Pharm., 69, 587.

EXERCISE V.—TANNINS

(Dissolve a little tannin in hot water or use the 1 per cent. solution.)

1. Add drop of Fe_2Cl_6 : green-blue-black color. Dilute until it is transparent. Add a few drops of NaOH: garnet color. Add cautiously an excess of H_2SO_4 : greenish-red; with more, greenish-yellow.

2. Add some $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$: large white precipitate. Add NaOH and shake: pink.

3. (Optional) Add some NaOH: reddish-brown color.

4. (Optional) Observe that tannin precipitates alkaloids (e. g., quinin), proteins (egg-white solution), and gelatin.

5. Add a drop of Fe_2Cl_6 to a little *infusion of Cinchona* (greenish color). The tannins occurring naturally in plants give a greenish color with iron; tannins occurring in pathologic formations (nutgalls) give a bluish color.

6. (Optional) **Gallic Acid.**—To a 1 per cent. solution of gallic acid add a few drops of 1 per cent. KCN: a red color appears, which soon fades, but reappears on shaking (Young's test). Pure tannic acid does not give this reaction.

QUESTIONS

(a) How would you test for tannins in a plant-extract?

(b) What groups of substances should not be prescribed in solutions with tannins?

(c) Why does tannin stop local bleeding?

(d) Why are tannin preparations useful in diarrhea?

S. M.—Rhubarb infusion, 5 per cent.

S. M.—Cinchona infusion, 5 per cent.

EXPLANATORY NOTES

Ferric Chlorid as Group Reagent.—Ferric chlorid gives color reactions with a number of organic drugs; for instance:

Red, with antipyrin; aliphatic amido-acids; meconic acid.

Violet, with apomorphin; nirvanin; salicyl compounds; resorcin; phloroglucin; phlorrhizin.

Blue, with morphin; phenol; cresols; naphthol; hydroquinon; gallic acid; phenol-sulphonic acids.

Green, with thallin; oxyquinolin; laudanin; epinephrin; pyrocatechin.

TECHNICAL REFERENCES

Isolation and testing of tannins, Abderhalden's Handb., 2, 996; 6, 146; *Determination in plant juices*, *ibid.*, 8, 259.

EXERCISE VI.—GUMS

(Use a 10 per cent. solution of acacia.)

1. **Hydrolysis by Acids.**—Test for sugar: negative. Add $\frac{1}{2}$ volume of 5 per cent. sulphuric acid, and boil for ten minutes in water-bath. Make alkaline with NaOH, and test for sugar: positive. This test is given in common by gums, starch, glucosids, and other carbohydrates.

2. Add some **alcohol**: **precipitate** (difference from glucosids; **borax** and **ferric chlorid** also cause precipitation or gelatinization).

3. Add a few drops of **iodin** solution: no blue color (difference from starch).

4. Note the viscosity of the solution; on shaking, it forms a rather persistent foam. It emulsifies oils, although less readily than saponin.

QUESTIONS

- (a) What are the characteristic properties of gums?
- (b) Why are gums incompatible with tinctures?
- (c) What would be the best menstruum for the extraction of gums?
- (d) What menstruum would be used to obtain extracts free from gums?
- (e) Explain the effect of gums on foaming and emulsification.

TECHNICAL REFERENCES ON CARBOHYDRATES

Abderhalden's Handb., 2, 43, 85, 119; 5, 1385, 1408; 6, 1; *Starch*, *ibid.*, 6, 1; *Soluble Starch*, *ibid.*, 6, 20; Samec and Jencic, Koll. Beih., 7, 137, 1915; *Sugars*, Abderhalden's Handb., 2, 43, 85; 5, 1385, 1408; quantitative methods, *ibid.*, 2, 167; in blood, *ibid.*, 5, 172; Lewis and Benedict, 1915, Jour. Biol. Chem., 20, 61; Kahn, 1915, Jour. Amer. Med. Assoc., 64, 241; *Cellulose*, Abderhalden's Handb., 6, 28; in feces, 5, 378; *Inulin*, *ibid.*, 6, 33; *Dextrin*, isolation, *ibid.*, 3, 218; *Levulose*, estimation in presence of glucose, Loewe, 1916, Soc. Exp. Biol. Med., 13, 71.

EXERCISE VII.—RESINS

(Use commercial rosin.)

1. **Solubility.**—Note that this is soluble in alcohol, but is precipitated from this solution by adding water. It is also soluble in ether, turpentine, fixed oils, and boiling sodium hydrate solution (precipitated by acids), but insoluble in gasolin.

S. M.—10 per cent. acacia.

QUESTIONS

- (a) State the important solubility characters of resins.
- (b) What would be a good menstruum for the extraction of resins from drugs?
- (c) Should resinous tinctures be mixed with waters?
- (d) Explain the actions of alkalies and acids on resins.
- (e) What would be the nature of the precipitate produced by nitric acid in the urine of a patient taking resin of copaiba?
- (f) How could you distinguish this precipitate from albumin?

EXERCISE VIII.—VOLATILE OILS

(Use oil of turpentine.)

1. **Solubility.**—Note that this mixes with alcohol, ether, gasolin, and cotton-seed oil, but not with water. Camphor, which may be considered as a solid volatile oil, behaves similarly.
2. Note that it makes a **greasy stain** on paper, but that this stain disappears in time, especially on heating.

QUESTIONS

- (a) State the solubility characters of volatile oils.
- (b) Are volatile oils absolutely insoluble in water?
- (c) What would be good menstrua for their extraction?
- (d) What occurs if "spirits" are mixed with waters?
- (e) How would you distinguish a volatile from a fixed oil?

TECHNICAL REFERENCES

Polarimetric estimation of Camphor in Spirit, etc., Jahrb. Pharmacie, 69, 354; *Determination of Camphor in urine*, Abderhalden's Handb., 3, 975; *Preparation of Volatile Oils*, *ibid.*, 2, 982.

EXERCISE IX (OPTIONAL).—CHLOROPHYLL

1. Note the green color of a fresh tincture of lettuce leaves.¹
2. Add some dilute HCl: yellow color.
3. To another portion add some NaOH: the color becomes an old gold-green. (Chlorophyll has a characteristic spectrum, in which the above reagents produce definite changes; see Hatcher and Sollmann; *Materia Medica*.)

TECHNICAL REFERENCES

Abderhalden's Handb., 2, 671; *Preparation*, Stanck, Chem. Abstr., 7, 1784; *Lipochrome*, Abderhalden, 2, 723, 758; *Animal Pigments*, *ibid.*, 2, 717.

FURTHER TECHNICAL REFERENCES ON PLANT CONSTITUENTS

Vegetable Proteins.—Abderhalden's Handb., 2, 270; *Animal*, *ibid.*, 335; *Removal*, *ibid.*, 1, 686.

Extract in Vegetable Preparations.—U. S. P. IX; Abderhalden's Handb., 8, 171.

Moisture.—U. S. P. IX; Abderhalden, 8, 167.

Methods of Rapid Desiccation of Tissues, etc.—Abderhalden's Handb., 5, 614; Wiechowski, 1907, Beitr. chem. Physiol., 9, 232; Shackell, 1905, Amer. Jour. Physiol., 24, 325; Beebe and Burton, 1905, *ibid.*, 14, 9; Rosenbloom, 1913, Jour. Biol. Chem., 14, 27; Lumiere and Chevrolier, 1912, Chem. Abstr., 7, 1521.

Melting Point.—U. S. P. IX.

Boiling Point.—U. S. P. IX.

Congeeing.—U. S. P. IX.

Solubility.—U. S. P. IX.

Ash.—U. S. P. IX; *Analysis*, Abderhalden's Handb., 1, 372; 5, 200, 1049; 6, 376.

Colorimeters.—Abderhalden's Handb., 1, 642; Roberts, 1910, Hyg. Bul. No. 66.

Color Standards.—Army and Ring, 1915, Jour. Amer. Pharm. Assoc., 4, 2294.

¹ Some fresh lettuce is bruised in a mortar with sand, triturated with alcohol, and filtered.

CHAPTER II

PHARMACEUTIC PREPARATIONS AND DISPENSING

Two students can collaborate on the experiments except those marked "individual." To save time the solids may be weighed in advance by the instructor. Some of the preparations will extend over several laboratory days. The student should always start the day's work with these unfinished preparations. The finished preparations should be submitted to the instructor, and can then be preserved in stock-bottles (for use in the later exercises). The formulas of the optional preparations can be found in the U. S. P. or N. F.

EXERCISE I.—AROMATIC WATERS

1. **Aqua Cinnamomi.**—In a dry mortar triturate 1 drop of cinnamon oil with about 0.5 gm. of talc; then add, gradually and with continued trituration, 25 c.c. of water. Pass repeatedly through a filter until the filtrate is perfectly clear.

Optional Preparations.—2. By filtration similar to the cinnamon: Aq. Camphoræ, Peppermint.

3. By simple solution: Aq. Chloroformi, Creosoti.

4. By distillation: Aq. Anisi.

QUESTIONS

- (a) Define the "aromatic waters."
- (b) What is the object of the talc?
- (c) What other methods could be used for making aromatic waters?

EXERCISE II.—LIQUORS

1. **Liquor Calcis.**—Slake 3 gm. of quicklime in an evaporating dish by the gradual addition of 100 c.c. of distilled water. Stir occasionally during half an hour. Let settle; decant the supernatant fluid and reject it. Rinse the insoluble residue into a bottle with 900 c.c. of distilled water; shake thoroughly; let stand twenty-four hours or longer. Shake again; let the coarser particles subside and pour the fine suspension into another bottle. Let stand, and pour off the clear "lime-water" as needed.

Optional Preparations.—2. By simple solution: Liq. Iodi Co.; Ac. Arsen.; Dobell's Solution; Hypodermic Injections; Ampouls.

3. By chemic processes: Liq. Ammon. Acet.; Chlori Co.; Ferri Chlor.; Magn. Citr.; Plumbi Subacet.; Potas. Arsenit.

QUESTIONS

- (a) Define a "liquor."
- (b) Explain the steps of the process for lime-water.
- (c) Why is it necessary to use distilled water?

EXERCISE III.—SYRUPS, ELIXIRS, GLYCERITES, MUCILAGES

1. **Syrupus.**—Heat 42.5 gm. of granulated sugar with 22.5 c.c. of water until dissolved; boil; strain through cloth, adding through the strainer sufficient water to make 50 c.c. (when cold).

S. M.—Cinnamon oil.

S. M.—Quicklime in 3-gm. portions.

Optional Preparations.—2. Prepared by adding the medicinal substance to syrup: Syr. Ac. Citrici; Rhei.

3. Prepared by dissolving sugar in the medicinal liquid: Syr. Ferri Iod.; Picis Liq.; Pruni Virg.

4. Elixir Aromaticum.

5. Glyceritum Boroglycerini.

6. Mucilago Acaciæ.

QUESTIONS

- (a) Define "syrups."
- (b) How do they differ from "elixirs"? (c) From "glycerites"? (d) From "mucilages"?
- (e) Why is the syrup boiled?

EXERCISE IV.—SPIRITS, COLLODIA

1. **Spiritus Menthæ Piperitæ.**—In a bottle dissolve 1 c.c. of oil of peppermint in 9 c.c. of alcohol; add 0.1 gm. of peppermint herb; macerate for twenty-four hours or longer, and filter.

Optional Preparations.—2. Spirits by simple solution: Spir. Ammon. Arom.; Camphor.
3. Collodia: Simple and Flexible.

QUESTIONS

- (a) Define "spirits."
- (b) How do they differ from "waters"?
- (c) How from "tinctures"?
- (d) What is the object of the peppermint herb?
- (e) What is a "collodion"?
- (f) How is collodion made flexible?
- (g) Under what circumstances would simple and flexible collodion be employed?

EXERCISE V.—INFUSIONS AND DECOCTIONS

1. **Infusum Digitalis.**—Crush 1.5 gm. of digitalis leaves in a mortar. Pour on to this 50 c.c. of boiling water. Let stand one hour. Strain through cloth. Add 15 c.c. of cinnamon water; and, through strainer, cold water sufficient to make 100 c.c.

Optional Preparations.—2. Barley Water: Wash 1 ounce of pearl barley; boil for short time with $\frac{1}{2}$ pint of water. Decant and throw out the liquid. Add to residue 4 pints of boiling water, boil down to 2 pints and strain.

QUESTIONS

- (a) Define "infusions" and "decoctions."
- (b) How do they differ from "solutions"?
- (c) From "tinctures"?
- (d) What is their strength when not specified?
- (e) What is the strength of infusion of digitalis?

EXERCISE VI.—TINCTURES, FLUIDEXTRACTS, SOLID EXTRACTS, OLEO-RESINS, RESINS

1. **Tinctura Arnicæ (by Maceration).**—Crush 10 gm. of arnica in a mortar. Transfer to a flask. Add 25 c.c. of official dilute alcohol (equal volumes of alcohol and water). Cork the flask and shake.

S. M.—Peppermint oil; peppermint herb in 0.1-gm. portions.

S. M.—Digitalis in 1.5-gm. portions; cinnamon water.

S. M.—Arnica in 10-gm. portions; cinchona, powdered, in 20-gm. portions.

After a week strain through cloth and express strongly. To the residue again add 25 c.c. of dilute alcohol, let stand a week and express. (Officially, two portions of 12.5 c.c. a day apart.) Mix the liquids.

2. **Tinctura Cinchonæ (by Percolation).**—Prepare a small percolator: pack a little cotton loosely in the neck; over this pour an inch of sand (Fig. 1).

Mix 7.5 c.c. of glycerin with 67.5 c.c. of alcohol and 25 c.c. of water. In an evaporating dish moisten 20 gm. of finely powdered cinchona uniformly with 8 c.c. of this menstruum. Transfer to the prepared percolator, without pressing. Let it stand well covered for an hour (preferably six hours).

Then pack it firmly (with the handle of the spatula) and pour on enough of the menstruum to saturate the powder and leave a stratum above it. When the liquid begins to drop from the percolator close the lower orifice, and let the tightly closed percolator macerate for forty-eight hours (or until the next laboratory period).

Then let the percolation proceed slowly (about 10 drops per minute), pouring on the remainder of the menstruum, and then enough of an alcohol-water mixture (67.5 A : 25 W, volume) until 100 c.c. of percolate are obtained.

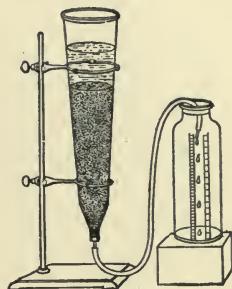


Fig. 1.—Method of percolation (Thornton).

Optional Preparations.—3. Tinctures by dilution: Tr. Ferri Chlor.; Nuc. Vom.

4. Tinctures by maceration: Tr. Cardam. Co.

5. Tinctures by percolation: Tr. Aconiti; Digitalis; Gentian. Co.; Opii; Opii Deod.

6. Fluidextracts: Ergot; Glycyrrhiza; Rhubarb; Wild Cherry; Senna.

7. Solid extracts: Cascara Sagrada; Rhubarb; Gentian; Ergot.

8. Oleoresins: Capsicum.

9. Resins: Podophyllum.

QUESTIONS

- What are the advantages of maceration and percolation?
- Why is the maceration of the arnica conducted in two or three stages?
- Why is it necessary to moisten the cinchona before placing it in the percolator?
- Why is it necessary to allow the percolator to stand two days before beginning the percolation?
- Explain the differences between tinctures, fluidextracts, solid extracts, oleoresins, and resins.

EXERCISE VII.—MIXTURES

1. **Mistura Cretæ Co.**—In a mortar mix prepared chalk (creta præparata), 3 gm.; acacia, 2 gm.; powdered sugar, 5 gm. (This makes “compound chalk powder.”) Rub this mixture with 4 c.c. of cinnamon water and 2 c.c. of water, gradually added, until a uniform mixture is obtained. Transfer to a graduate and rinse the mortar with enough water to make 10 c.c.

2. **Simple Suspension of Chalk.**—Rub 3 gm. of prepared chalk with 7 c.c. of water. Compare the permanence of this suspension with the preceding.

S. M.—Cinnamon water.

3. **Bismuth Mixture.**—Make a mixture of bismuth subcarbonate, acacia, cinnamon water, and water. There are to be 3 tablespoon doses; each dose is to contain 0.5 gm. each of bismuth subcarbonate and of acacia, and equal parts of cinnamon water and water.

Optional Preparations.—4. *Lotio Nigra*; *Lot. Plumbi et Opii*; *Magma Magnesiae*.

QUESTIONS

- (a) Define a mixture.
- (b) Why is it necessary to add acacia or sugar to suspensions of heavy powders?

EXERCISE VIII.—EMULSIONS

1. **Emulsum Olei Morrhuae** (Official "Continental" Method).—In a dry mortar rub 10 c.c. of cod-liver oil with 2.5 c.c. of finely powdered acacia to a uniform smooth mixture. Then add at once 5 c.c. of water and triturate lightly and rapidly until a thick homogeneous emulsion is produced. To this add 2 c.c. of syrup and 3 c.c. of water. (The official emulsion is flavored with 0.4 per cent. of wintergreen oil.)

Optional Preparations.—2. *Emuls. Asafet.*; *Emuls. Turpent.*

3. **Lecithin Emulsions.**—Dissolve 5 gm. of Merck's lecithin in 50 c.c. of water and triturate with 45 gm. of the oil (Bloor, 1913, *Jour. Biol. Chem.*, 15, 112).

4. *Casein Emulsion.*—Raper, 1913, *ibid.*, 14, 117.

QUESTIONS

- (a) Define an emulsion.
- (b) What are the proportions for making the "nucleus"?
- (c) How does the gum act in helping the subdivision and suspension of the oil?
- (d) What other substances act as emulsifying agents?
- (e) How can the thin (volatile) oils be emulsified?
- (f) How are emulsions of gum-resins (*asafetida*) made?

EXERCISE IX.—LINIMENTS

1. **Linimentum Calcis** ("Carron Oil").—Shake thoroughly 10 c.c. of lime-water (calcium hydrate solution) and 10 c.c. of cotton-seed oil (officially, raw linseed oil).

Optional Preparations.—2. *Liniments of Ammonia*; *Camphor*; *Chloroform*; *Turpentine*.

QUESTIONS

- (a) Define a liniment.
- (b) What are the usual bases of liniments?
- (c) What is formed in the preparation of the lime liniment?

EXERCISE X.—POWDERS

1. **(Individual) Powder Papers.**—Divide 10 gm. of starch into 10 powders, properly folded, as demonstrated.

Optional Preparations.—2. *Compound Powders*: *Compound Effervescent Powder*; *Compound Licorice Powder*.

3. *Effervescent Salts*: *Effervescent Magnesium Sulphate*.

4. *Chemic Compounds*: *Saccharated Carbonate of Iron*; *Precipitated Sulphur*.

S. M.—Cod-liver oil; syrup.

QUESTIONS

(a) Why should compound powders be triturated in a definite order, proceeding from the ingredient of the smallest to that of the largest bulk?

(b) What physical properties render a substance unsuitable for powder papers?

(c) Why is it inadvisable to dispense a dose of less than 5 grains alone in powders? How could this difficulty be overcome?

EXERCISE XI.—PILLS

1. (Individual) **Glycyrrhiza Pills.**—Place 2 gm. of powdered glycyrrhiza in a mortar and incorporate excipient (glycerite of acacia) a little at a time, sufficient to form a plastic mass. Care must be used lest too much excipient is added (this may be remedied by adding a little dry acacia). The mass must be worked very thoroughly, until it can be rolled in the hand without breaking.

Dust the pill tile with a little starch, and on it roll out the mass to a uniform cylinder extending over 10 or 20 divisions. Dust the spatula with starch, and with it divide the cylinder into 10 exactly equal parts. Roll each part to a spherical pill between the thumb and first two fingers. Place the pills into the lid of the pill box, with a little starch, and roll them perfectly round with the ball of the thumb.

Pills must be of uniform size, smooth shape, and sufficiently firm to resist gentle pressure. Several trials should be made if necessary.

Optional Preparations.—2. Pills of Aloes; Ferrous Carbonate; Silver Nitrate.

3. Tablet Triturates; Compressed Tablets; Lozenges; Suppositories.

QUESTIONS

(a) What are the functions of the excipient?

(b) What other excipients are used in pills?

(c) What are the drawbacks of pills?

(d) What classes of substances should not be prescribed as pills?

(e) What are the ordinary limits to the size of pills?

EXERCISE XII.—CAPSULES

1. (Individual) **Starch Capsules.**—Divide 2 gm. of starch into 10 parts, and place in capsules ("No. 3"), as demonstrated. Roll the finished capsules in the hand (or better, clean cloth) to remove adherent powder.

Optional Preparations.—2. "Soft" capsules of castor oil.

3. "Ampouls" (Proc. Amer. Pharmaceut. Assoc., 57, 53).

QUESTIONS

(a) What are the advantages and disadvantages of capsules as compared with pills?

(b) What are the ordinary limits to the weight of capsules?

EXERCISE XIII.—OINTMENTS

1. (Individual) **Unguentum Zinci Oxidum.**—In a dry mortar rub 2 gm. of zinc oxid with 10 gm. of benzoinated lard, gradually added, until they are

S. M.—Powdered glycyrrhiza in 2-gm. portions.

S. M.—No. 3 capsules.

S. M.—Zinc oxid in 2-gm. portions; benzoinated lard in 10-gm. portions.

thoroughly mixed and free from lumps. Or they may be mixed in a pill tile; the zinc being placed at one side, the lard on the other, and the two being gradually worked together in the middle by a spatula.

Optional Preparations.—2. Simple ointment; ointments of phenol; boric acid; sulphur; tar; yellow mercuric oxid.

QUESTIONS

- (a) Why is it essential that the ointment be free from lumps?
- (b) What are the relative advantages and disadvantages of lard, petrolatum, and wool-fat?

EXERCISE XIV.—POULTICES

1. **Cataplasma Lini.**—Boil 50 c.c. of water and a small pinch of sodium bicarbonate; stir into this gradually ground flaxseed (*linum contusum*) until a thick mush is obtained (about 50 gm.).

Optional Preparation.—2. Spreading a plaster.

QUESTIONS

- (a) Why is the linseed added to the water and not vice versa?
- (b) Why is the soda added?

CHAPTER III

INCOMPATIBILITY

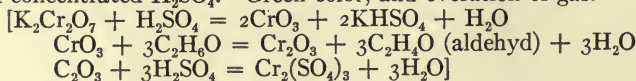
It is assumed that the student, through analytic chemistry, is familiar with most of the reactions which underlie incompatibility. Only a few of these are reviewed here as types. They should be performed by each student individually. The criticism of the incompatible prescriptions will need some aid from the instructor. They may be compounded as optional experiments.¹

EXERCISE I.—EXPLOSIVES

1. **(Optional) Potassium Chlorate.**—Rub a trace of the chlorate and tannin in a mortar: detonation.

2. **(Optional) Nitric Acid.**—Mix some strong nitric acid and alcohol in a beaker, and let stand under a bell jar: in a short time orange vapors arise, and suddenly the solution boils up and is thrown from the beaker.

3. **Chromates.**— $\text{K}_2\text{Cr}_2\text{O}_7$ solution + Alcohol: no change. (There may be a slight precipitate, which redissolves if a little water is added.) Add concentrated H_2SO_4 . Green color, and evolution of gas.



S. M.—Ground flaxseed.

¹ An extensive compilation of incompatibilities is contained in Ruddiman's "Incompatibilities in Prescriptions," New York, 1908.

QUESTIONS

- (a) What other substances would explode when rubbed with tannin?
 (b) With chlorate?
 (c) In what order should the ingredients of the following gargle be mixed to avoid explosion?

R.	KClO ³	0.5	} If put up without heating, no change will occur, illustrating the possibility of mixing certain explosives in solution.
	Aquæ.....	10.0	
	Glycerini.....	2.0	
	Tr. Ferri Chlor.....	1.0	

- (d) In what order should they be mixed if the gargle is to contain free chlorin?
 (e) What salts oxidize organic matter even in dilute solution?

CRITICIZE THE FOLLOWING PRESCRIPTIONS

- | | | |
|--|--|---|
| (a) Potas. Nitrate.
Sulphur. | (b) Silver Nitrate.
Cocain.
Water. | (c) Tr. Iodin.
Ammon. Chlorid.
Water. |
| (d) Potas. Permang.
Glycerin.
Water. | (e) Silver Nitrate.
Tap Water. | |

EXERCISE II.—IODIDS

1. **Liberation of Iodin by Oxidizing Agents.**—Mix solutions of potassium iodid and hydrogen peroxid: brown color. ($2\text{KI} + \text{H}_2\text{O}_2 = 2\text{KOH} + 2\text{I}$.)

2. **Precipitation of Metallic Salts.**—(a) Mix solutions of potassium iodid and lead acetate: yellow precipitate.

(b) Mix solutions of potassium iodid and mercuric chlorid: red precipitate, soluble in excess of either reagent.

(c) Mix solution of potassium iodid and a little calomel: yellow color (mercurous iodid), gradually changing to green, gray or black, by decomposition into metallic mercury and mercuric-potassium iodid.

3. **Precipitation of Strychnin.**—To 10 c.c. of KI solution (5 per cent.) add 10 drops of 1 per cent. strychnin sulphate. Keep until crystals of strychnin iodid develop (if necessary until next laboratory period).

QUESTIONS

- (a) What other substances evolve I from KI?
 (b) What other metals precipitate with iodids?
 (c) What metals are precipitated by bromids?
 (d) By chlorids?
 (e) Explain the changes with KI and HgCl₂.
 (f) Why is it dangerous to administer calomel to a patient receiving iodids?
 (g) Are most alkaloids precipitated by KI?
 (h) By HBr?

CRITICIZE THE FOLLOWING PRESCRIPTIONS

- | | | |
|---------------------------------------|---|---|
| (a) NaI.
Sp. Ether Nitr.
Water. | (b) Tr. Iodin.
Potas. Permang.
Water. | (c) KI.
Liq. Potas. Arsenitis.
Water. |
| (d) Silver Nitrate.
Normal Saline. | | |

S. M.—Strychnin sulphate 1 per cent.

EXERCISE III.—ALKALIES

1. **Precipitation of Earths.**—(a) Mix solutions of magnesium sulphate and sodium carbonate: white precipitate of magnesium carbonate.

(b) Mix solutions of magnesium sulphate and sodium bicarbonate: no precipitate.

2. **Precipitation of Metals.**—(a) Mix solution of sodium bicarbonate and tincture of ferric chlorid: evolution of CO_2 and precipitation of brown ferric carbonate.

(b) Mix solutions of ferric ammonium citrate and sodium bicarbonate: no precipitate.

(c) Mix solutions of alum and sodium borate: white precipitate of aluminum hydroxid.

(d) Mix solutions of alum and boric acid: no precipitate.

3. **Precipitation of Alkaloids.**—(a) Mix liq. potas. arsenitis with a few drops of saturated quinin sulphate: precipitate of quinin. Add a few drops of dilute hydrochloric acid: solution.

4. **Decomposition of Chloral.**—Mix solutions of chloral and sodium hydroxid: odor of chloroform. ($\text{CCl}_3\text{COH} + \text{NaOH} = \text{NaCHO}_2 + \text{CHCl}_3$.)

5. **Decomposition of Hexamethylenamin by Acids.**—To a solution of hexamethylenamin add dilute HCl and heat: odor of formaldehyd. ($(\text{CH}_2)_6\text{N}_4 + 6\text{H}_2\text{O} = 4\text{NH}_3 + \text{CH}_2\text{O}$.)

QUESTIONS

- (a) Are all salts of earths precipitated by alkalies?
- (b) Why does not the bicarbonate precipitate the magnesium?
- (c) Does it also prevent the precipitation of the metals?
- (d) Are the salts of all metals precipitated by alkalies?
- (e) Why is the double citrate not precipitated?
- (f) Would the sodium borate precipitate alkaloids?
- (g) How could the precipitation of alkaloids by the arsenite be prevented?
- (h) Which salts act as alkalies?

CRITICIZE THE FOLLOWING PRESCRIPTIONS

- | | | |
|--------------------|------------------|-----------------------|
| (a) Liq. Calcis. | (b) Magn. Sulph. | (c) Tr. Nuc. Vom. |
| Sod. Bicarb. | Sod. Phosph. | Sp. Ammon. Arom. |
| | | Elix. Arom. |
| (d) Bism. Subnitr. | (e) Syr. Scillæ. | (f) Hexamethylenamin. |
| Sod. Bicarb. | Ammon. Carb. | Ammonium Chlorid. |
| Water. | | |

EXERCISE IV.—SALICYLATES

1. **Precipitation by Acids.**—Mix solution of sodium salicylate with dilute hydrochloric acid: white precipitate of salicylic acid.

2. **Color with Iron.**—To a solution of sodium salicylate add a few drops of ferric chlorid: violet color of ferric salicylate.

3. **Precipitation of Quinin.**—Mix solutions of quinin sulphate (saturated) and sodium salicylate: white precipitate of quinin salicylate.

QUESTIONS

- (a) Why is sodium salicylate usually given with sodium bicarbonate?
- (b) What other substances give colored solutions with iron?
- (c) Do most alkaloids precipitate with salicylates?

CRITICIZE THE FOLLOWING PRESCRIPTIONS

- | | | |
|------------------------------------|---|---|
| (a) KI.
Sod. Salicyl.
Water. | (b) Sp. Æth. Nitr.
Sod. Salic.
Water. | (c) Sod. Salic.
Antipyrin.
Make a powder. |
|------------------------------------|---|---|

EXERCISE V.—TANNIN

1. **Precipitation of Metals.**—Mix solutions of tannin and mercuric chlorid: white precipitate of mercuric tannate.

2. **Precipitation of Alkaloids.**—Mix solutions of quinin sulphate (saturated) and tannin: gray precipitate of quinin tannate. Add alcohol: solution.

QUESTIONS

- (a) Are all metals precipitated by tannin?
- (b) What other change occurs with ferric salts?
- (c) Are all alkaloids precipitated by tannin?
- (d) Are glucosids precipitated by tannin?
- (e) What other substances are precipitated by tannin?

CRITICIZE THE FOLLOWING PRESCRIPTIONS

- | | | |
|--------------------------------------|---|--|
| (a) Tr. Ferri Chlor.
Tr. Cinchon. | (b) Liq. Potas. Arsen.
Ac. Tann.
Water. | (c) Ac. Tann.
H ₂ O ₂ .
Water. |
| (d) Gelatin.
Tannin.
Water. | | |

EXERCISE VI.—PHARMACEUTIC INCOMPATIBILITY

1. **Alcoholic Preparations and Water.**—(a) Mix Sp. Ammon. Arom. and water: precipitate of oils.

(b) Mix Tr. Myrrh and water: precipitate of the resins.

2. **Alcohol and Water-soluble Drugs.**—(a) Mix Muc. Acacia with alcohol: precipitate of the gum.

(b) Mix Sat. Sol. Sod. Chlorid with alcohol: precipitate of the salt. Add water: solution.

3. **Solubility.**—Mix 1 part liquefied phenol with 10 parts of water. How can this be brought into solution?

QUESTIONS

- (a) Are all alcoholic preparations incompatible with water?
- (b) Why is the salt precipitated by the alcohol?

CHAPTER IV

ISOLATION OF POISONS

The isolation of poisons by the students themselves requires more time than can be usually given in this course. It would also require very considerable experience before their results would be practically trustworthy. The exercises of this chapter are therefore presented as demonstrations. A good presentation of Toxicologic Analysis is given in Gadamer's "Lehrbuch der chemischen Toxicologie," Goettingen, 1909; in Abderhalden's Handb., 5, 673; and in Autenrieth, 1915.

S. M.—Sp. ammon. arom.; tr. myrrhæ; mucil. acaciæ.

EXERCISE I.—(DEMONSTRATION) VOLATILE POISONS

A mixture of meat or similar material, poisoned with phenol (or hydrocyanic acid, chloral, alcohol, etc.), is diluted with water, acidulated with tartaric acid, and distilled from a flask through a Liebig condenser. The distillate has the characteristic odor of the substance and may be subjected to the corresponding tests.

EXERCISE II.—(DEMONSTRATION) DISTILLATION TEST FOR PHOSPHORUS

The poisoned material is placed in a flask connected with a steam kettle and vertically descending Liebig condenser (Fig. 2) arranged in a dark room. The air is expelled from the flask by steam; the flask is then heated. The characteristic luminous ring appears in the tubes or condenser, shifting

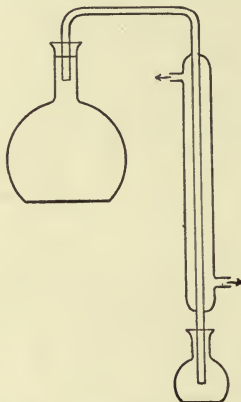


Fig. 2.—Mitscherlich apparatus.

its position according to the heat applied. The presence of other volatile substances interferes with the test.

EXERCISE III.—(DEMONSTRATION) ISOLATION OF FIXED ORGANIC POISONS BY MODIFIED STAS-OTTO METHOD

The instructor should perform the experiment in advance, preparing the various stages of the separation; so that only the steps of the process need be demonstrated, without halting the demonstration to wait for the separation to take place.

1. **Extraction.**—To a mixture of 30 gm. of hashed meat and 3 gm. of powdered nux vomica add about 100 c.c. of water and a pinch of tartaric acid. Boil for ten minutes. Cool. Strain through Canton flannel. Reject the solid residue.

2. **Removal of Salts, Proteins, and Fats.**—Add about 10 gm. of sand (or some purified oak saw-dust) to the strained solution, and evaporate, first on free flame, then on water-bath, to a paste. Add 40 c.c. of 95 per cent. alcohol, let stand ten minutes or longer, with frequent stirring; and filter. The salts, proteins, and fats are left on the filter, since they are insoluble in alcohol. These are rejected. The alcoholic solution contains the organic poisons.

3. **Removal of Resins, Fats, etc.**—Dilute the alcoholic solution with an

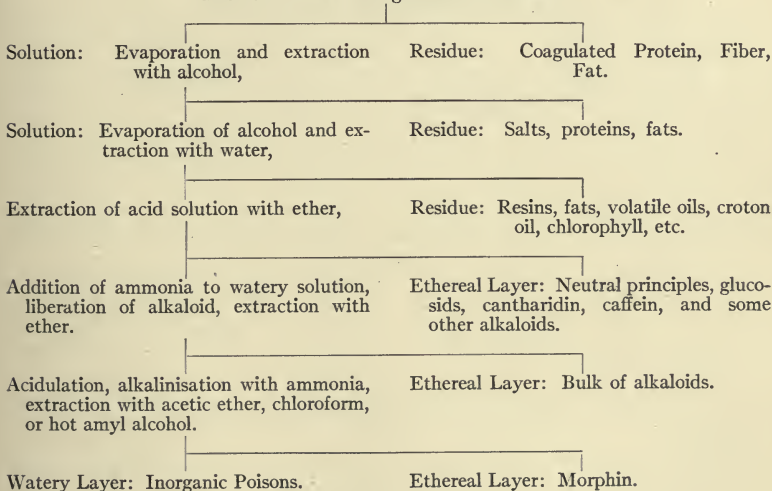
equal volume of water. This precipitates the above impurities (active resins and croton oil would be found in this precipitate). Filter. Reject the precipitate. Evaporate to near dryness to remove the alcohol. Dissolve the residue in 50 c.c. of water. Filter. Assure yourself that the filtrate is acid.

4. **Removal of Neutral Principles and Some Other Impurities.**—Place the solution in a separating funnel, add 25 c.c. of ether, and shake with a gentle rotatory motion for ten minutes. Separate the two layers. The ethereal layer would contain the neutral principles, which could be obtained by evaporating the ether. In the present instance the ethereal layer is rejected. The watery layer contains the alkaloidal salts. It is treated by (5).

5. **Extraction of Alkaloids.**—Replace the watery solution of 4 in the separating funnel. Add ammonia until it is freely alkaline (this liberates the free alkaloids, which are soluble in ether. The alkaloidal salts are insoluble and were, therefore, not extracted in 4). Add 25 c.c. of ether and shake with a rotatory motion for ten minutes. Let the liquid separate, and draw off the watery layer (which would contain morphin); this is rejected. The ethereal layer contains most of the alkaloids. Distil off the ether. Test some of the residue for Strychnin and Brucin. Dissolve another portion in a little dilute sulphuric acid, inject into a frog, and note the convulsions. (The ether extractions would be repeated, in practice, as long as they would take up any alkaloid.)

Explanatory Note.—The method rests on the different solubility of the constituents of the mass in successive solvents. It may be represented diagrammatically as follows:

Extraction with boiling dilute tartaric acid.



TECHNICAL NOTES

Emulsification during extraction is often a very disturbing occurrence. It is less liable to occur if the shaking is done with a very gentle rotatory motion. Various means for its avoidance are described in the U. S. P. IX. La Wall, 1914, advocates a special type of separatory funnel to avoid emulsification (Jour. Amer. Pharm. Assoc., 3, 498). The theory of immiscible solvents is discussed by Gadamer, 362.

EXERCISE IV.—(DEMONSTRATION) DESTRUCTION OF ORGANIC MATTER FOR ISOLATION OF INORGANIC POISONS BY FRESenius-BABO METHOD

Organic matter more or less obscures the reactions of inorganic poisons and must, therefore, be destroyed. The Fresenius-Babo method¹ is generally preferred.

(A) Place the material (meat, etc., poisoned with arsenic) in a liter flask with as much arsenic-free HCl as would correspond to the dry material. Dilute with sufficient water to make a thin gruel. This is heated luke-warm on a water-bath, and potassium chlorate added at intervals, 0.5 gm. at a time, until the material is practically dissolved (not more than 4 to 6 gm. should be used). The solution is then boiled in an evaporating dish to 100 c.c., or until free from chlorine, diluted to 400 c.c., 2 c.c. of dilute sulphuric acid are added, and the mixture is set aside over night, and filtered. The filtrate (B) would contain most of the metals; the residue (K) would contain Ag, Ba, and Pb. The further separation is effected by the schema given below. Only the test for arsenic need be demonstrated.

Marsh's Test (see B).—Produce hydrogen in flask by acting on pure zinc with arsenic-free HCl; pass through CaCl_2 , then through tubes drawn

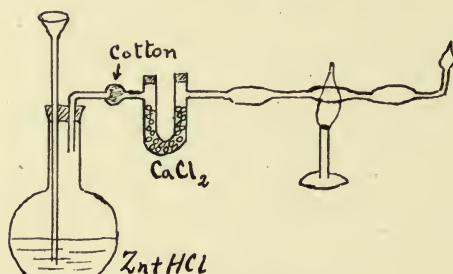


Fig. 3.—Marsh apparatus.

out at several places (Fig. 3). Heat to redness at the thick portion of a segment. (This blank test should be continued for six hours.) If no mirror appears, introduce the suspected solution. Black mirror occurs with arsenic or antimony. They may be distinguished as follows:

ARSENIC:

Mirror beyond heated portion.
Garlic odor on heating in air.
Dissolves in hypochlorite.
Easily volatilized when heated in hydrogen.

Heated in air, yields easily volatilized white crystals.

Heated in H_2S , yellow, insoluble ble in HCl.

Dissolved in HNO_3 , evaporated, plus AgNO_3 , plus vapor of NH_3 , red or yellow precipitate.

ANTIMONY:

Mirror *at* head portion.

No odor.

Not.

Not easily volatilized.

Amorphous white residue, not easily volatilized.

Red (black on strong heating); soluble in HCl.

No color in cold; black (metallic Ag) on heating.

¹ Further details, Gadamer, 110.

SCHEMA FOR ISOLATION OF METALS

Filtrate B.—Pass through filter water to just 500 c.c. Use 50 c.c. for Marsh's test. If *As* is present, use remainder for quantitative (see *C*). If not, evaporate small sample, dissolve in 10 c.c. water, add NH_4OH : blue = *Cu*.

C.—Heat remainder of filtrate B to 80°C . and pass arsenic-free H_2S for two or three hours, until cool. Heat again, and repeat. Stopper and set aside in warm place for twenty-four hours. *Precipitate* may contain *As*, *Sb*, *Hg*, *Cu*, *Pb*. It may be used for the quantitative estimation of *As*, or for further identification by *D*. *Filtrate* = *I*.

D (*H_2S precipitate of C*).—Wash with H_2S water, warm with 4 c.c. ammon. sulphid, 4 c.c. ammonia, 8 c.c. water. Filter. *Filtrate* = *E*; *Insoluble* = *F*.

E (*Filtrate of D*).—Evaporate to dry; heat with HNO_3 until pure yellow; heat to expel HNO_3 ; add Na_2CO_3 and NaNO_3 ; fuse; extract with boiling water; add 2 gm. NaHCO_3 ; filter; *Filtrate* contains *As* and may be used for quantitative. The *insoluble* = *Sb*: apply tests.

F (*Insoluble of D*).—Oxidize residue and filter in capsule with HCl and KClO_3 ; filter; dilute; heat; pass H_2S ; filter; wash precipitate with warm HNO_3 . *Filtrate* = *G*. *Precipitate* = *H*.

G (*Filtrate of F*).—Add 10 drops dilute H_2SO_4 ; evaporate; take up with water. *Residue* = *Pb*; *Filtrate* = *Cu*. (Apply tests.)

H (*Precipitate of F*).—Oxidize with aqua regia; evaporate; filter; dilute; test for *Hg*.

I (*Filtrate of C*).—Use half for zinc, half for chromium.

Zn: Neutralize with KOH ; acidulate with $\text{H}_2\text{C}_2\text{O}_3$; precipitate with H_2S ; wash precipitate with $\text{H}_2\text{C}_2\text{O}_3$ in H_2S water (1 : 5); incinerate, precipitate, and filter; dissolve in dilute H_2SO_4 , plus a little HNO_3 ; evaporate dry; dissolve in H_2O ; test for *Zn*.

Cr: Evaporate to just moist; mix with KNO_3 ; dry; fuse; dissolve; test for *chromate*.

K (*Residue of A*).—Fuse with KNO_3 , Na_2CO_3 , and NH_4NO_3 . Suspend in H_2O ; pass CO_2 ; boil; filter. Dissolve precipitate in dilute HNO_3 . Test this solution for *Ag*, *Ba*, and *Pb*.

Electrolytic Determination of Metals.—Directions are given in the U. S. P. IX and in Gadamer, 130.

EXERCISE V.—(OPTIONAL) ALKALOIDAL ASSAY

The U. S. P. process for Belladonna is typical of the majority of assays (with the important exception of opium). It consists in a modification of Keller's method. The quantitative estimation of alkaloids is also described in Gadamer, 496; Abderhalden's Handb., 6, 120; Autenrieth (Warren, 1915), pp. 86 and 246; and in the monograph of von Korczynski, "Methoden der exacten quantitativen Bestimmung der Alkaloide," Berlin, 1913.

EXERCISE VI.—(OPTIONAL) PHARMACEUTIC TESTING

The U. S. P. tests for purity are well illustrated by the following:

1. Sodium Bromid.
2. Time limit test for heavy metals.
3. Iron Sulphate.
4. Acetphenetidin.
5. Quinin Sulphate.
6. Chloroform.
7. Ether.

TECHNICAL REFERENCES

Determination of Melting Point.—U. S. P. IX; Abderhalden's Handb., 1, 208; Menge, 1910, Hyg. Lab. Bul. No. 70.

Boiling Point.—U. S. P. IX; Abderhalden, 1, 214; Small quantities, Gadamer, 271; for molecular weight, Abderhalden, 6, 364.

Solubility Determination.—U. S. P. IX; Abderhalden, 1, 451; Seidell, 1910, Hyg. Lab. Bul. No. 67.

QUESTIONS ON CHAPTER IV

- (a) Why is it necessary to acidulate the material before the distillation of volatile poisons?
- (b) Why is an organic acid used rather than a mineral acid?
- (c) Why is it advantageous to conduct the distillation with live steam?

- (d) In testing for phosphorus, why must the air be expelled from the flask before heating?
- (e) Does the failure of the luminous ring-test exclude phosphorus?
- (f) What is the general principle of the extraction of fixed organic poisons?
- (g) Why is the material boiled with acidulated water?
- (h) Would this be necessary when working on urines?
- (i) How would the process be modified in this case?
- (j) How would it be modified if the suspected poison is difficultly soluble in water?
- (k) In the ethereal extraction, would it make any difference whether 30 c.c. of ether is used at one time, or in three 10 c.c. fractions?
- (l) Why is it necessary to destroy the organic matter when searching for mineral poisons?
- (m) How is this accomplished?
- (n) Why would it be inadvisable simply to incinerate the material?
- (o) State the principle of the Marsh test.

CHAPTER V

SPECIAL TESTS OF IMPORTANT ALKALOIDS

The main object of these exercises is to familiarize the student with the reactions which are utilized in toxicologic analysis and in the urine, food, etc. It must be remembered that impure products give these tests very imperfectly. They may, however, be applied to tablets,¹ capsules, etc., especially if these are first extracted with suitable solvents. When the dry substance is used, the reaction is performed on a glass slide or watch-glass, placed on white paper; or on a porcelain slab. A piece of broken evaporating dish may be used if the reaction requires heat. A mere trace of the substance, about a milligram, should be employed. When solutions are used, the reactions are generally made in a test-tube or capsule. The student should remember that he is handling very strong poisons.

The tests need not be memorized, but should be described in the notes or checked in the book. Two students may work together. The physiologic tests are stated for convenience of reference, but need not be performed at this time.

EXERCISE I.—STRYCHNIN

(Physiologic test: peculiar convulsions in frogs or mice.)

To a trace of the powdered alkaloid add:

1. A drop of concentrated H_2SO_4 : no change; then a small crystal $\text{K}_2\text{Cr}_2\text{O}_7$. Play of colors through blue, violet, red, orange (Otto).
2. A drop of concentrated NHO_3 ; heat gently: with most samples a yellow color, due to Brucin.
3. Determine the dilution at which the bitter taste of strychnin just disappears (begin with 1 : 50,000; to 5 c.c. of this add water in portions of 1 c.c.). Report your results. (The usual limit is 1 : 40,000 to 1 : 67,000. Should any student depart markedly from this, he should try his sensitiveness to other bitter substances.)

¹ *Analysis of Tablets*, Kebler, 1914, Jour. Amer. Pharm. Assoc., 3, 6, 107.

4. (Optional).—Strychnin, even in very dilute solutions, gives a white precipitate with chlorin water.

5. (Optional) Isolation of Strychnin.—Proceed as in Chapter IV, Exercise III, but in (5) use chloroform as solvent.

6. (Optional) Piconolic Acid for Purification of Strychnin and Other Alkaloids.—This was proposed by W. H. Warren and Weiss (Jour. Biol. Chem., 3, 330, 1907). The piconolate of strychnin being very insoluble, may be precipitated from aqueous solution, thus separating it from other substances that interfere with purification.

7. (Optional) Quantitative Determination.—Salant, 1904, Jour. Med. Res., 12, 51.

8. (Optional) Determination of Strychnin in Tablets.—Kebler, 1914, Jour. Amer. Pharm. Assoc., 3, 1098.

9. (Optional) Brucin.—This is important mainly because of its association with strychnin in *nux vomica*.

(a) To a little of the powdered alkaloid add a small drop of nitric acid: blood-red color. Add a few drops of 1 per cent. sodium thiosulphate (hyposulphite): violet color (Cotton).

(b) To some powdered *Nux Vomica* add a drop of concentrated HNO_3 ; orange color, due to Brucin.

EXERCISE II.—CAFFEIN

1. Moisten some powdered alkaloid with nitric acid: yellow to orange color. Evaporate the excess of acid on water-bath and expose to ammonia vapor: garnet to purple color (murexid reaction of Stenhouse, Rochleder). (Theobromin and theophyllin give very similar reactions.)

2. (Optional) Isolation of Methylxanthins.—Proceed as in Chapter IV, Exercise III, 1 to 4. Then extract the *acid* solution with chloroform, which dissolves the methylxanthins.

3. (Optional) Isolation from Urine.—The acid urine is shaken directly with chloroform, which dissolves the methylxanthins, but not the normal urinary xanthin bases.

TECHNICAL REFERENCES

Quantitative Estimation.—Off. Agric. Chem., Abderhalden's Handb., 2, 610; 6, 132; Preparation, Abderhalden, 2, 959.

Coffee, Tea, and Chocolate, ibid., 7, 373; *Detection of Chicory* in Coffee decoctions, La Wall, Amer. Jour. Pharm., 85, 535.

Theobromin and Theophyllin, Preparation and tests, Abderhalden's Handb., 2, 610, 960.

EXERCISE III.—MORPHIN

1. To a solution (about 1 : 1000) of morphin sulphate add a little fresh sodium iodate solution, a few drops of dilute sulphuric acid, and a little starch-paste: purple color. This is a very delicate test, but is also given by other reducing substances (Mohr).

2. To a little (2 per cent.) *aqueous* solution in a test-tube add a drop of (neutral) ferric chlorid: blue color (Schaer); not delicate.

3. To a trace of powdered alkaloid add a drop of nitric acid and heat: orange color.

4. To a trace of dry alkaloid add a drop of fresh Marquis' (Kobert's) reagent (concentrated H_2SO_4 , 20 c.c.; 40 per cent. formalin, 1 c.c.). Play of colors from purple-red to violet blue.¹

5. Mix a trace of dry alkaloid with an equal quantity of ammonium molybdate, and add a drop of concentrated sulphuric acid (Froehde's reagent): violet color, changing to deep blue.

S. M.—Strychnin sulphate solution, 1 : 50,000.

S. M.—Starch paste; Marquis' reagent; ammon. molybdate; $\frac{1}{8}$ gr. morphin tablets.

¹This reagent gives somewhat similar reactions with phenols and their derivatives (carbolic acid, salicylic acid, resorcin, etc.). (Optional experiments.) The color in the cold is, however, more pink than with morphin, carbolic acid being the only one which could give rise to a mistake. This can be removed by boiling the acidulated solution until it ceases to give the phenol reactions (Hatcher).

6. To a few drops of (2 per cent.) aqueous solution in a test-tube add about 2 c.c. of concentrated HCl and a few drops of concentrated H_2SO_4 . Boil in water-bath for one-half hour: apomorphin is formed. Neutralize with Na_2CO_3 (solution) and add a drop of Tr. Iodin: emerald color. Shake with ether: this takes a violet color (Pellagri's reaction—also given by codein, heroin, etc.).

7. **Morphin in Tablets, etc.**—(a) Dissolve $\frac{1}{8}$ -grain tablet in a few drops of water, and apply Tests 1 and 2.

(b) Crush another tablet, shake with chloroform and a drop of ammonia; filter; evaporate on three watch-glasses; apply Tests 4 and 5.

8. (Optional) **Quantitative Estimation in Tablets.**—Rep. Chem. Lab. Amer. Med. Assoc., 1913, 6, 88 (precipitation by ammonia); Kebler, 1914, Jour. Amer. Pharm. Assoc., 3, 1093; in *Tablets and Pills*, H. W. Jones, 1915, Jour. Amer. Pharm. Assoc.

9. (Optional) **Isolation of Morphin from Tissues, etc.**—Proceed as in Chapter IV, Exercise III, using chloroform or amyl alcohol in No. 5.

10. (Optional) **Quantitative Isolation.**—Ruebsamen, 1910, Arch. exp. Path. Pharm., 64, 54; Kaufmann-Asser, 1913, Bioch. Zs., 54, 161.

Physiologic Test.—Erection of mouse-tail.

TECHNICAL REFERENCES

Further tests for Morphin.—T. H. Oliver, 1914, ref., Jour. Amer. Med. Assoc., 63, 513.

Isolation from Tissues.—Gadamer, 551; G. L. Schaefer, 1913, Amer. Jour. Pharm., 85, 439; Cloetta, 1903, Arch. exp. Path. Pharm., 50, 455; Thorburn, 1911 (Phenyl ethyl alcohol), Jour. Ind. Eng. Chem., 3, 754; Girard, Deléarde and Ricquet, Bioch. Centr., 4, 451. *Plomains* do not interfere, Rosenbloom, 1914, Jour. Biol. Chem., 18, 131.

Quantitative Estimation.—Abderhalden's Handb., 6, 126; Gadamer, 551; Sanger and Broughton, 1909 (adaptation of Marquis' test), Proc. Soc. Biol. Chem., 1, 250; Gordin and Harrison, 1906 (in presence of glycerin), Jahrb. Pharm., 66, 308.

Preparation of Opium Alkaloids.—Abderhalden, 2, 942.

EXERCISE IV.—CODEIN, HEROIN, AND RELATED ALKALOIDS

Codein and heroin, as all esters of morphin, give the Pellagri reaction (Exercise III, No. 6) for apomorphin. They do not give the reactions 1 and 2.

Special reactions are as follows:

1. (Optional) **Codein.**—Place a little of the dry alkaloid in a capsule and add a few drops of concentrated H_2SO_4 : faint greenish, then violet, color. Add a drop of concentrated HNO_3 : plays from yellow to purple.

2. (Optional) **Heroin.**—A little of the dry alkaloid is dissolved in a watch-glass in a few drops of nitric acid: yellow color; on standing or heating this turns greenish blue and then fades again to yellow.

3. (Optional) **Acetyl Radicals of Heroin.**—Heat a trace of heroin with dilute sulphuric acid in a test-tube, add some alcohol and boil: acetic odor.

4. (Optional) **Dionin.**—This gives most of the reactions of codein, but somewhat different colors with Marquis' reagent.

5. (Optional) **Isolation of Codein, Dionin, Peronin, and Heroin.**—Codein, dionin, and peronin are extracted from the alkaline solution by ether (Chapter IV, Exercise III), using sodium carbonate in place of ammonia in No. 5. The extraction of heroin is as for morphin.

6. (Optional) **Narcotin.**—To a trace of powdered alkaloid add some concentrated sulphuric acid: greenish yellow solution, turning to orange, intensified on heating. On continued heating, violet with purple streaks (Arnold).

7. (Optional) **Papaverin.**—L. E. Warren, 1915, Jour. Amer. Chem. Soc., 37, 2402.

8. **Meconic Acid (Serving as a Test for Opium).**—Dilute a few drops of tinct. opii with water and add drop of ferric chlorid: red color, not bleached by HgCl_2 .

S. M.—Tr. opii.

9. **Apomorphin.**—(a) To a trace of dry alkaloid add a drop of nitric acid: blood-red color.

(b) To a few drops of (about) 1 : 500 watery solution (note the green color) add 5 drops of Na_2CO_3 and a drop of alcoholic iodine: emerald color. Shake with ether. This becomes violet.

(c) (Optional).—To 5 drops of apomorphin solution add 5 drops of saturated solution of mercuric chloride; then 5 drops of 10 per cent. sodium acetate. Boil for a few minutes, cool, and add 1 to 2 c.c. of amyl alcohol. This is colored blue on shaking. The test is extremely delicate (to 1 : 500,000, Amer. Jour. Pharm., 87, 564, 1915).

(d) (Optional).—Apply test (b) to apomorphin tablets.

(e) (Optional).—*Presence of Apomorphin in Morphin.*—To a dilute solution of the hydrochloride add 3 drops of 1 per cent. potassium ferrocyanide, and shake with benzol. If apomorphin is present, the benzol acquires an amethyst color. On shaking with NaOH, this turns reddish violet, deepening to violet on standing (sensitive to 0.003 mg., Feinberg, 1913, Zs. physiol. Chem., 84, 363).

10. (Optional) **Hydrastin.**—(a) Dissolve in dilute sulphuric acid and add dilute potassium permanganate: blue fluorescence (hydrastinin).

(b) To the dry alkaloid add concentrated sulphuric acid: no color; heat: violet.

(c) *Isolation.*—As per Chapter IV, Exercise III, 1 to 5.

(d) *Hydrastin in Fluidextract Hydrastis.*—Shake 5 drops of the fluidextract with 5 c.c. of 5 per cent. sodium bicarbonate and 10 c.c. of ether. Wash the decanted ether layer with 5 c.c. of water. Filter the decanted ether layer and evaporate it to dryness. Dissolve the residue in 10 c.c. of dilute sulphuric acid, and add 12 to 15 drops of 1 : 1000 potassium permanganate. The solution is decolorized, but after dilution with 5 volumes of water it shows a blue fluorescence in reflected light (Glueckmann, 1913, Pharm. Post., 348).

11. (Optional) **Berberin.**—(a) Note yellow color of solutions, even when very dilute.

(b) To a solution add chlorin water: red color (Klunge).

(c) To a solution add solution of KI: precipitate.

(d) *Quantitative.*—Richter, W., 1914, Arch. Pharm., 252, 192; ref., Zentr. Bioch. Bioph., 17, 476.

(e) *Berberin in Fluidextract Hydrastis.*—To 10 c.c. of concentrated hydrochloric acid add a drop of the fluidextract, shake, add a drop of hydrogen dioxide solution, and shake again: a persistent red color develops in five to ten minutes (Glueckmann, 1913, Pharm. Post., 348).

TECHNICAL REFERENCES

Heroin Tests.—Zernick, 1903, Jahrb. Pharm., 339—in excreta, Langer, 1912, Bioch. Zs., 45, 222; *Rapid Determination* of small quantities, R. Miller, 1915, Amer. Jour. Pharm., 87, 248. *Separation of Heroin and Morphin*, Doran, 1916, Jour. Amer. Pharm. Assoc., 5, 163.

Tests for Minor Opium Alkaloids.—L. E. Warren, 1915, Amer. Jour. Pharm., 87, 437.

Apomorphin Preparation.—Abderhalden's Handb., 2, 956; Purity tests, Chem. Abstr., 5, 1497.

Oxydimorphin, Reagent, Hoshida, 1908, U. S. P. Digest, 353. Tests in presence of morphin, Grimbirt and Leclerc, 1914, ref., Zentr. Bioch. Bioph., 18, 625.

Hydrastis Alkaloids, Preparation.—Abderhalden, 2, 945.

EXERCISE V.—COCAIN AND ANESTHETIC BASES

Physiologic Test for Cocain.—Local anesthesia and dilation of pupils.

1. (Optional) **Cocain.**—(a) To a solution of cocain hydrochloride on a slide add some 1 per cent. potassium permanganate: characteristic violet crystals.

(b) Triturate some cocain with an equal (about) quantity of calomel; moisten with dilute alcohol: turns gray by reduction of mercury (Flueckiger). Dilute with a little water and boil. Fruity odor of methyl benzoate.

(c) Add a crystal of cocain to solution of alpha-naphthol in 40 per cent. KOH: blue color.

(d) *Isolation of Cocain.*—As per Chapter IV, Exercise III, Nos. 1 to 5.

Quantitative Determination.—Rifatwachdani, 1913, Bioch. Zs., 54, 83 (also Ecgonin).

2. (Optional) **Distinction of Cocain and Substitutes.**—Seiter and Enger, 1911, Amer. Jour. Pharm., 83, 195; Gadamer, 576.

S. M.—Apomorphin HCl 1 : 500 solution.

TECHNICAL REFERENCES

Demonstration of Cocain.—Vardan, 1908, Bioch. Central., 8, 169; Hankin, Jahrb. Pharm., 71, 194.

Preparation.—Abderhalden's Handb., 2, 929.

EXERCISE VI.—ATROPIN AND RELATED ALKALOIDS

The following tests are given by all the solanaceous mydriatic alkaloids and their derivatives:

1. Place a trace of dry atropin in a test-tube. Add 10 drops of concentrated H_2SO_4 , and heat until it becomes brown; then add 2 volumes of water: characteristic odor, resembling tuberose (Gulichno), strengthened by KMnO_4 (Reuss).

2. (Optional) **Vitali's Reaction.**—In an evaporating dish heat some of the dry alkaloid with a few drops of fuming nitric acid to dryness. Moisten the yellow residue with alcoholic KOH: reddish-violet color.

3. (Optional) **Presence of Apotropin in Atropin or Scopolamin.**—To a solution of the suspected alkaloid add a drop of 1 per cent. permanganate: the presence of apotropin causes an immediate reduction (brown precipitate). Pure atropin or scopolamin remain clear (Kessel, 1906, Arch. intern. Pharmacod., 16, 1).

4. (Optional) **Distinction of Belladonna Bases.**—This is made by the characters of their gold salts.

5. (Optional) **Isolation.**—Proceed by Chapter IV, Exercise III, using sodium bicarbonate in No. 5.

6. (Optional) **Solanin.**—Preparation, Abderhalden's Handb., 2, 966; Tests, *ibid.*, 6, 133.

Physiologic Tests.—Dilation of pupils and paralysis of vagi.

TECHNICAL REFERENCES

Preparation of Atropin, Abderhalden's Handb., 2, 921; *Quantitative recovery of atropin from tissues*, Fickewirth and Heffter, 1913, Bioch. Zs., 40, 37; *Preparation of Scopolamin*, Abderhalden, 2, 927.

EXERCISE VII.—EPINEPHRIN, PHYSOSTIGMIN, PILOCARPIN, NICOTIN, AMINS, AND RELATED ALKALOIDS

1. **Epinephrin.**—(a) Dilute solutions turn pink or brown on standing. This is hastened by alkalis.

(b) To some 1 : 50,000 solution of epinephrin, or to a dilute extract of suprarenal gland, add some ferric chlorid, drop by drop, as long as the color darkens: a green color develops. Add some NaOH: the color changes to a dark brownish red (Vulpian's Chromogen Reaction).

Physiologic Tests.—Vasoconstriction; dilation of pupils; inhibition of intestines or uterus.

TECHNICAL REFERENCES

Other Chemic Tests for Epinephrin.—Comesatti (mercuric chlorid), *ref.*, Jour. Amer. Med. Assoc., 51, 1474; Henle's microchemic test, Elliott, Brit. Med. Jour., July 15, 1905.

Quantitative Methods.—Seidell, 1913, Jour. Biol. Chem., 15, 197, 1914, Hyg. Lab. Bul. No. 100; Folin, Cannon and Denes, 1913, Jour. Biol. Chem., 13, 477; Vanderkleed, 1906, Jahrb. Pharm., 66, 263; Hale and Seidell (Krauss), Chem. Abstr., 7, 804, 1913, U. S. P. IX.

2. (Optional) **Physostigmin.**—(Notice pinkish color.) (a) To 1 : 1000 aqueous solution add 1 drop of NaOH: red; becomes green on heating, and returns to red on cooling. Add Sulphurous Acid: again colorless (Eber).

(b) Evaporate some solution with a few drops of NH_3 : red color, leaving dry blue residue. Add water: blue solution. Add Acetic Acid: violet in transmitted, coppered fluorescent in reflected, light.

S. M.—Atropin.

S. M.—Epinephrin, 1 : 50,000.

Physiologic Test.—Constriction of pupil.

3. (Optional) **Pilocarpin.**—Shake some dry pilocarpin hydrochlorid with a granule of potas. dichromate, 2 c.c. of chloroform, and 1 c.c. of 3 per cent. H_2O_2 . The chloroform acquires a blue or violet color. Apomorphin, strychnin, and antipyrin give rather similar but distinct reactions.

4. (Optional) **Nicotin.**—(a) Equal volumes of ethereal solutions of nicotin and iodine give a precipitate, changing gradually to large red needles (Roussin's crystals).

(b) *Estimation in Tobacco.*—Off. Agric. Chem.

Physiologic Tests.—Frog-position, tremors, vagus ganglia.

TECHNICAL REFERENCES

Preparation of Pilocarpin.—Abderhalden's Handb., 2, 963; of *Hordein*, *ibid.*, 965; *Piperin*, *ibid.*, 917; *Sparteine*, *ibid.*, 932; *Coniin*, *ibid.*, 909; *Arecolin*, *ibid.*, 914.

Estimation of Nicotin in Tobacco, etc.—Assoc. Off. Agr. Chem.; Abderhalden, 2, 916; 6, 128.

Isolation of conium alkaloids from animal tissues, Dilling, 1909, *Bioch. Jour.*, 4, 286.

Cholin, Preparation and Tests.—Abderhalden's Handb., 2, 522; Renshaw, 1910, *Jour. Amer. Chem. Soc.*, 32, 128; Rosenheim, 1905, *Jour. Ph.*, 33, 220; Kaufmann and Vorlaender, 1910, *Zentr. Bioch. Bioph.*, 11, 3; *Physiologic Test*, R. Hunt, 1915, *Jour. Pharmacol. Exp. Ther.*, 7, 307; *Isolation*, Stanek, 1906, *Zs. physiol. Chem.*, 47, 83; 48, 334; *Estimation*, Kinoshita, 1910, *Arch. ges. Physiol.*, 132, 607; Ellinger, 1914, *Muench. Med. Woch.*, 2336.

Betain.—Abderhalden's Handb., 2, 522; 7, 74.

Cytisin.—Preparation, Abderhalden, 2, 968.

Cod-liver Oil Bases.—*Ibid.*, 2, 1042.

Amin Bases.—Preparation, *ibid.*, 8, 261.

"*Simpler Natural Bases.*"—Barger, 1914.

Vitamins.—Isolation, Sullivan and Voegtlin, 1916, *Proc. Amer. Soc. Biol. Chem.*, 3, 16.

EXERCISE VIII.—ACONITIN, VERATRIN, COLCHICIN

1. **Aconitin.**—(a) *Prickling Taste.*—Note the taste of aconite (1 : 300) perceptible in dilution of 1 : 600 when 4 c.c. of the dilution is kept in the anterior part of the mouth for one minute. This has been used for quantitative estimation.

(b) (Optional).—There are no characteristic chemic tests for pure aconitin, but the commercial samples generally give the following test for Pseudoaconitin (Vitali): Evaporate the alkaloid with fuming nitric acid on water-bath, and moisten with alcoholic KOH: red color, tinged with violet.

Physiologic Test.—Frog's heart.

2. **Veratrin (Cevadin).**—To a trace of powdered alkaloid add:

(a) A drop of concentrated H_2SO_4 : yellow color. Apply heat: the color changes through orange and deep scarlet to a beautiful violet red.

(b) A drop of concentrated HCl and heat: red color (Trapp).

Physiologic Test.—Peculiar action on muscle.

3. (Optional) **Colchicin.**—To a trace of the powdered alkaloid add a drop of concentrated sulphuric acid: yellow solution. Add a trace of nitric acid: green, blue, violet, yellow. Make alkaline with KOH: yellowish red (Other chemic and physiologic tests, Fuehner, 1910, *Arch. exp. Path. Pharm.*, 63, 357; Reichard, 1912, *Yearb. Amer. Pharm. Assoc.*, 1, 417).

EXERCISE IX.—QUININ

Use a saturated aqueous solution of quinin sulphate.

1. Notice the blue fluorescence, best seen by drawing the solution into a pipet. This is increased by acids, diminished by NaCl.

S. M.—Aconite, 1 : 300.

2. **Thalleioquin Reaction.**—Add 2 drops of bromin water (enough to give a permanent precipitate), and then cautiously an excess of ammonia. An emerald color results, which is changed to red by HCl. (If a very small quantity of ammonia is used, the color may be magenta.) (Brandes, André.)

3. (Optional) **Herapathite Reaction.**—To an alcoholic solution of quinin add some iodine reagent (1 part iodine, 1 part 50 per cent. HI, 50 parts 70 per cent. alcohol, 0.8 part sulphuric acid). Let stand: Crystalline plates, with green metallic luster, polarizing strongly.

4. (Optional) **Quinin in Tablets.**—Extract with a little water and apply Reaction 2.

5. (Optional) **Determination in Urine.**—See Nishi, 1909, Arch. Exp. Path. Pharm., 60, 318; Baldoni, 1912, ref., Zentr. Bioch. Bioph., 14, 315; *ibid.*, 17, 837; Abderhalden's Handb., 3, 942.

TECHNICAL REFERENCES

Thalleioquin Reaction.—Fuehner, 1905, Arch. Pharm., 244, 602; technic, Abensaur, Bioch. Centr., 6, 551.

Quantitative Estimation of Quinin.—Abderhalden's Handb., 6, 125; Dufilho, 1914, Zentr. Bioch. Bioph., 16, 885.

Cinchonin in Urine.—Abderhalden, 3, 943.

Preparation of Cinchona Alkaloids.—*Ibid.*, 2, 934.

Watson's Test for Cinchona Alkaloids.—Add a few drops of alcoholic alpha-naphthol containing 2 drops of concentrated sulphuric acid per 1 c.c. The cinchona alkaloids (and no others) give a yellow precipitate, soluble in an excess of the reagent (Yearb. Amer. Pharm. Assoc., 2, 418, 1913).

QUESTIONS ON CHAPTER V

- (a) Describe a characteristic test for strychnin, morphin, and quinin.
- (b) How would you isolate an alkaloid from a hypodermic tablet?

CHAPTER VI

SPECIAL TESTS FOR IMPORTANT GLUCOSIDS AND NEUTRAL PRINCIPLES

EXERCISE I.—(OPTIONAL) DIGITALIS PRINCIPLES

1. **Kiliani's Test.**—Two solutions are used: (A) 100 c.c. concentrated sulphuric acid with 1 c.c. of 5 per cent. ferric sulphate. (B) 100 c.c. glacial acetic acid with 1 c.c. of 5 per cent. ferric sulphate. The digitaloid is dissolved in 3 to 4 c.c. of (B), and under this is poured an equal volume of (A), and allowed to stand.

Digitoxin gives a dark contact zone and deep blue acetic layer.

Digitalin (true) colors the sulphuric acid yellow, red, and finally reddish violet.

Digitonin (pure) gives no color.

2. **Keller's Test for Digitoxin.**—Dissolve in glacial acetic acid containing a little ferric chlorid. Float this on strong sulphuric acid: result as in 1.

3. **Strophanthin.**—(a) K-strophanthin (official): Moisten the dry substance with 80 per cent. sulphuric acid: green color.

This test is also given directly by the seeds of *Strophanthus Kombé* and *hispidus*. However, it disappears with storage (Baldoni, 1915, Arch. di Farm., 19, 511).

(b) H-strophanthin: Moisten with concentrated sulphuric acid: red color.

(c) G-strophanthin (ouabain): Dissolve in a little water and pour on concentrated sulphuric acid: acid pink to red; water dirty green.

4. **Digitonin** (and "German Digitalin") has the characters of saponin, laves blood, and forms a characteristic compound with cholesterolin.

Physiologic Test for Digitaloids.—Frog's heart slowed, and systolic standstill.

TECHNICAL REFERENCES

Chemic tests for Digitalis constituents, Kiliani, 1913, Amer. Jour. Pharm., 85, 223;
 Dimethylamidobenzaldehyd as chemic test, Bufalini, 1913, Arch. Farmacogn., Sept. 15.
Isolation of Digitoxin from organs, etc., Gadamer, 433.

EXERCISE II.—(OPTIONAL) SANTONIN AND EMODIN CATHARTICS

1. **Santonin Color Reactions.**—(a) Dissolve a little in alcohol, add a small piece of dry KOH, and warm: reddish-green to yellow color (Banfi).

(b) To a trace of the dry substance add a little concentrated sulphuric acid and a drop of ferric chlorid, and heat: dark red color, changing to violet brown.

(c) Rub a small quantity with KCN and heat: dark red mass, dissolving in water or alkalies with green fluorescence.

2. **Isolation from Tablets.**—Extract with chloroform, evaporate, and apply the tests.

3. **Isolation from Feces or Gastric Contents.**—Heat on water-bath with milk of lime for several hours; strain; shake with benzol to remove impurities. Acidulate with HCl and extract with chloroform or benzol. Purify by crystallization from hot water or by cautious sublimation between watch-glasses.

4. **Santonin Urine.**—None of the santonin appears unchanged in the urine, but occurs there as *Santogenin*, probably as a combination of mono- and dioxysantonin (Jaffe, 1897, Zs. physiol. Chem., 22, 538). The urine polarizes to the left, and is yellow when acid, red when alkaline.

5. **Distinction from Rhubarb and Similar Urines.**—The urine after chrysophanic acid, rhubarb, senna, and other emodin cathartics is also yellow when acid, red when alkaline. This may be distinguished from santonin by the following tests:

(a) Sodium carbonate colors the rhubarb urine at once, santonin only after a time. The red color is permanent with rhubarb, but disappears in one or two days with santonin.

(b) Lime-water precipitates the red color with rhubarb, not with santonin (Munk).

(c) *Digestion with zinc dust decolorizes the red rhubarb urine, not the santonin.*

(d) Ether shaken with the acid urine is colored yellow with rhubarb, unchanged with santonin. On adding alkali to the decanted ether layer, this turns red with rhubarb, but remains colorless with santonin (Penzoldt).

(e) Amyl alcohol shaken with the alkaline urine takes up the color from santonin, not from rhubarb (Hoppe-Seyler).

6. **Aloes in Urine.**—The urine is shaken in a test-tube with an equal volume of acetic ether. The ether is decanted and evaporated, the residue dissolved in a little alcohol, and a trace of copper sulphate added: red color.

TECHNICAL REFERENCES TO SANTONIN

Tests, C. Reichardt, 1907, U. S. P. Digest, 404; Determination in Santonica, C. E. Caspari, 1914, Jour. Amer. Pharm. Assoc., 3, 634.

Pelletierin.—Abderhalden's Handb., 2, 921.

EXERCISE III.—(OPTIONAL) PICROTOXIN

(a) Note the intensely bitter taste (one! drop of 1 : 1000 solution on tongue).

(b) Mix an equal quantity (trace) of picrotoxin and powdered potassium nitrate; add a drop of concentrated sulphuric acid, and then, drop by drop, a strong sodium hydrate solution: brick-red color (Langley's Reaction).

Physiologic Test.—Peculiar convulsions of frog.

EXERCISE IV.—(OPTIONAL) CANTHARIDIN

Abderhalden's Handb., 2, 889.

CHAPTER VII

SPECIAL TESTS OF IMPORTANT AROMATIC DERIVATIVES

EXERCISE I.—PHENOLS

The following tests are given in more or less modified form by all phenols, although the typical colors apply only to phenol proper:

1. **Phenol.**—Use a 5 per cent. solution.

(a) Add a trace of Fe_2Cl_6 : blue-violet color.

(b) Add bromin-water: yellow precipitate (tribromphenol) of needle-shaped crystal (Landolt).

(c) Add Millon's reagent (mercurous nitrate) and heat: blood-red color or precipitate (Plugge).

(d) (Optional) **Azo-dye Reaction.**—To a few cubic centimeters of 1 per cent. anilin hydrochlorid add a few drops of concentrated HCl; cool on ice; add a few drops of 5 per cent. NaNO_2 ; make alkaline with 15 per cent. NaOH, and add the alkaline phenol solution: brownish-yellow color. Acidulate with HCl: red precipitate.

(e) (Optional) **Isolation of Phenol.**—Acidulate with tartaric acid and distil with steam.

(f) (Optional) **Phenol Urines.**—These are distinguished by their smoky color; richness in ethereal and deficiency of inorganic sulphates; and sometimes contain free phenol.

(g) (Optional) **Phenol Estimation in Urine.**—Abderhalden, 3, 823; 5, 313; colorimetric, Folin and Denis, 1915, Jour. Biol. Chem., 22, 305; Phenol and Paracresol, Siegfried and Zimmermann, 1915, Bioch. Zs., 70, 124.

Methods of Phenol Estimation.—Forbing, 1916, Jour. Amer. Pharm. Assoc., 5, 166; Permanganate method, Pence, 1913, Jour. Ind. Eng. Chem., 5, 218; Determination of Phenol in cresol mixtures, Ditz and Bardach, 1912, Bioch. Zs., 37, 272.

(h) (Optional) **Phenol Estimation in Tissues.**—Sollmann, Hanzlik and Pilcher, 1910, Jour. Pharmacol., 1, 442; E. M. Mumford, 1913, ref., Yearb. Amer. Pharm. Assoc., 2, 382.

(i) Note that the reaction of strong carbolic acid to litmus paper is neutral.

(j) (Optional) **Phenyl-sulphonates.**—Barium chlorid does not precipitate directly, but does so after prolonged boiling with HCl.

2. (Optional) **Cresols.**—*Creosote*, *Guaiacol*, and *Thymol* give tests similar to those of phenol.

(a) (Optional) **Determination of Guaiacol in Urine.**—Boil the urine with HCl; shake with ether; evaporate ethereal layer, dissolve in alcohol, and test with trace of ferric chlorid: blue or green color.

(b) (Optional) **Isolation of Thymol from Urine.**—A. Seidell, 1915, U. S. Hyg. Lab. Bul. 101, 43.

3. (Optional) **Beta-naphthol.**—This also gives similar reactions. The following are distinctive: (a) It dissolves in alkalis with blue fluorescence.

(b) Dissolve in concentrated alkali, add a few drops of chloroform and heat: blue color (Lustgarten).

(c) In urine: to 5 c.c. of urine add 3 or 4 drops of solution of chlorinated lime and a few drops of concentrated HCl: lemon yellow color (naphthoquinon). Shake with ether: this takes up the color. Pour this over 1 per cent. aqueous resorcin: red ring.

4. (Optional) **Resorcin.**—This gives the usual phenol tests. (a) The azo-dye reaction is deep purple.

(b) It gives a pink color with NaOH and a trace of chloroform (Reuter).

(c) Isolation from urine: evaporate to one-quarter; boil with sulphuric acid; extract with ether; evaporate ethereal layer. The resorcin is in the ethereal layer, and may be purified with charcoal.

5. (Optional) **Pyrogallol.**—(a) Solutions are colored violet, brown, or black by lime-water.

(b) It reduces solutions of silver and other metals.

(c) With formaldehyd and concentrated HCl it gives a red color in the cold or on gentle heating.

6. *Indol Reaction*.—Baudisch, 1915, *Zs. physiol. Chem.*, 94, 133; *determination*, Cantelli, 1915, *ref., Zentr. Bioch. Bioph.*, 18, 59.

EXERCISE II.—ANILIN DERIVATIVES

1. **Common Tests**.—The anilin derivatives, of which acetanilid and acetphenetidin are the most important, give the indophenol reaction, which depends on the amido group

(a) *Indophenol Reaction*.—Boil some acetanilid (or phenacetin) with a little concentrated HCl for one or two minutes (to liberate the anilin and form paramidophenol). Cool; add an equal volume of 5 per cent. phenol (to form indophenol) and a few drops of fresh solution of chlorinated lime: red turbid fluid. Supersaturate with ammonia and shake: indigo blue color.

2. (Optional) **Distinctive Tests Between Acetanilid and Phenacetin**.—(a) Heat some acetanilid with NaOH solution: Dissolves, with odor of anilin; add a few drops CHCl_3 and heat again: Odor of phenyl-isonitril (resembles witch-hazel). This reaction is also given by anilin, but not by phenacetin, etc. (Hofmann.)

(b) Rub together equal volumes of Acetanilid and NaNO_2 and add some concentrated H_2SO_4 : Orange liquid. Phenacetin gives a violet black color, later passing into green.

(c) Boil with HCl and add a few drops of 3 per cent. chromic acid: acetanilid gives a yellow color changing to green; phenacetin, ruby red.

3. (Optional) **Isolation from Tablets, etc.**—Extract with ether, evaporate and apply the tests. *Quantitative Estimation*, Seidell, 1907, *Jour. Amer. Chem. Soc.*, 29, 1091; Kehler, *Jour. Amer. Pharm. Assoc.*, 3, 1078, 1914.

4. (Optional) **Isolation from Organs**.—Proceed by Chapter IV, Exercise III, Nos. 1 to 4. Watery extractions should be made hot.

5. (Optional) **Tests in Urine**.—These substances are excreted mainly as paramidophenol, and therefore give the indophenol reaction: To about 10 c.c. of urine add $\frac{1}{4}$ volume of concentrated HCl; boil; allow to cool; add $\frac{1}{8}$ volume of 5 per cent. carbolic acid and a few drops of potassium bichromate solution; red color: add ammonia: blue color.

6. (Optional) **Anilin**.—This gives the indophenol reaction without previous heating with HCl.

EXERCISE III.—ANTIPYRIN

1. To an aqueous solution add a few drops concentrated Fe_2Cl_6 : deep red solution; + H_2SO_4 : light yellow (Cohn, Knorr).

2. To an aqueous solution add some Spiritus Ætheris Nitrosi. Slow development of green color and precipitate of isonitroso-antipyrin.

3. (Optional) Antipyrin precipitates the alkaloidal precipitants.

4. (Optional) **Test in Urine**.—Apply Test 1 directly to the urine.

5. (Optional) **Determination in Tissues**.—Lauber and Winter, 1913, *ref., Chem. Abstr.*, 7, 1729.

EXERCISE IV.—SALICYL DERIVATIVES

These give the reactions of phenols.

1. **Sodium Salicylate**.—(a) To a dilute solution add a drop of dilute ferric chlorid: red violet color. (The reaction is hindered by acids.)

(b) Place some dry salicylate in test-tube; add equal parts of methyl alcohol and concentrated H_2SO_4 and heat: odor of methyl salicylate (oil of wintergreen).

S. M.—Chlorinated lime, fresh solution.

(c) (*Optional*) *Isolation from Tissues, Foods, etc.*—Either by distillation of the acid solution, or by extraction according to Chapter IV, Exercise III, Nos. 1 to 4.

Salicylic acid is often used as a *food preservative* (about 0.2 gm. per liter or kilo). It is detected by the ferric chlorid test (a), but it must first be isolated in fairly pure form. If the material is *solid* or *semisolid*, 200 to 300 gm. are hashed, triturated with 400 c.c. of slightly alkaline water, and strained. This liquid (or the original sample, if it be *liquid*) is acidulated with sulphuric acid and extracted with ether or chloroform, and the ethereal or chloroformic layer washed twice with a little water. If the sample contained *little or no fat*, this extract may be evaporated directly, at a low temperature, and the residue taken up with hot water. This may be divided into several portions and used also for the tests for benzoic acid and saccharin. Since fruits may contain salicylic acid, not more than 50 c.c. of wine or 50 gm. of fruit should be represented by the portion of the extract which is used for the salicylic test. If this quantity gives the test, one may be sure that salicylic acid has been added. Only 2 or 3 drops of 0.5 per cent. Fe_2Cl_6 should be used.

If the sample contains considerable fat, the ethereal or chloroformic solution is extracted with dilute ammonia-water, the ammoniacal watery solution evaporated almost to dryness, divided, and tested as above.

For the detection in milk, 500 c.c. of the milk and 50 gm. of sand are evaporated to dryness on a water-bath. The residue is extracted with acidulated alcohol. The alcoholic filtrate is neutralized with ammonia, evaporated to dryness, dissolved, divided, and tested as above.

2. (*Optional*) *Demonstration of Salicyl in Esters* (Wintergreen oil, salol, aspirin, etc.).—Some give the iron reaction directly; all do so after saponification.

To a solution of the ester in water or dilute alcohol (or to the distillate of the organs or extract) add a few drops of NaOH solution; boil a few minutes; add a drop of dilute ferric chlorid; acidulate lightly with HCl; cool; neutralize carefully with ammonia: violet color.

3. (*Optional*) *Determination of Salicylates in Urine.*—(a) Add a few drops of ferric chlorid: violet color. This test is generally sufficient.

(b) Acidulate the urine and shake out with ether. Decant the solvent, and shake it with very dilute ferric chlorid: violet color.

In place of the ether, a mixture of 3 parts of petroleum ether and 2 parts of chloroform may be used, which gives less emulsification; or a mixture of ether and benzol, which dissolves also the salicyluric acid.

Salicyl urines polarize to the left, and reduce Fehling's feebly.

TECHNICAL REFERENCES ON SALICYLIC ACID

Isolation from Foods, Milk, Tissues, etc.—Offic. Agric. Chem.

Quantitative Determination in Organs, etc.—Bondi and Jacoby, 1905, Beitr. Chem. Physiol., 7, 518; Seidell, 1909, Jour. Amer. Chem. Soc., 31, 1164; Cassal, 1910, Bioch. Centr., 10, 674; Bondzynski and Humnicki, 1909, Jahrb. Pharm., 69, 218; Sauerland, Bioch. Zs., 40, 65, 1912.

Quantitative Estimation in Urine.—Abderhalden's Handb., 3, 958; Gadamer, 336; bromin method, Lagrange, 1906, Paris Thesis.; Hanzlik, 1916.

Phenol Impurity.—Carletti, 1907, U. S. P. Digest, 127.

Salicyluric Acid Determination.—Baldoni, 1915, Arch. di Farm., 18, 1.

EXERCISE V.—BENZOIC ACID AND SACCHARIN

1. (*Optional*) *Benzoic Acid.*—(a) To dilute solution of sodium benzoate add drop of neutral ferric chlorid: brownish-pink precipitate. Add a little dilute HCl: dissolves. (A white precipitate of benzoic acid may be thrown down if the solution was concentrated.)

(b) *Conversion into Salicylic Acid.*—To 10 c.c. of 1 : 1000 benzoic acid add 3 drops of a 1 : 10 dilution of Liq. Ferri Chloridi, then 3 drops of 3 per cent. ferrous sulphate, shaking after each addition. A violet color develops in one-half to ten minutes; sensitive to 0.1 to 0.2 mg. (Jonescu; Fleury; ref., Jahrb. Pharm., 73, 170, 1913).

(c) *Isolation.*—Similar to salicylic acid. It may be separated from the latter by bromin water, which does not precipitate benzoic acid.

For the detection of benzoic acid used as a *food preservative* (0.5 gm. per kg. being the usual quantity); this is isolated by the methods described under Salicylic Acid.

For the final test, it is essential that only 1 or 2 drops of a 0.5 per cent. neutral Fe_2Cl_6 solution be employed.

TECHNICAL REFERENCES

Determination in Foods.—Offic. Agric. Chem., Hilger, 1909, Jour. Ind. Eng. Chem., 1, 538; Jonescu, Bioch. Centr., 8, 918; in catsup, La Wall and Bradshaw, Amer. Jour. Pharm., 80, 171; in milk, Leach, Jour. Pharm., 1903, 486; in butter, Reinsch, Jour. Pharm., 1903, 496.

Quantitative Estimation.—Folin and Flanders, 1911, Jour. Amer. Chem. Soc., 33, 161; in urine, Raiziss and Dubin, 1915, Jour. Biol. Chem., 20, No. 2.

In Urine.—Abderhalden's Handb., 3, 831.

2. (Optional) **Hippuric Acid.**—Determination in urine, Abderhalden's Handb., 3, 828; 5, 315; rapid, 7, 720; Folin and Flanders, 1911, Jour. Amer. Chem. Soc., 33, 161.

3. (Optional) **Saccharin.**—This is sometimes used as an adulterant in sweets, etc. It differs from sugars in being soluble even in ether and chloroform. If, therefore, the substance is extracted with these solvents, and this solution evaporated at a low temperature, a sweet taste of the residue indicates the presence of this adulterant. The extraction may be made in a separating funnel, the substance, if solid, being dissolved in water. A chemical test may be applied by heating this residue with NaOH to 210° C., which converts it into salicylic acid, characterized by the color reaction with iron. If salicylic acid was present originally, this must first be destroyed by oxidation with KMnO₄.

TECHNICAL REFERENCES

Abderhalden's Handb., 7, 356; *Determination in foods*, Offic. Agric. Chem.; Testoni, Jahrb. Pharm., 69, 425; *Estimation in urine and feces*, Bloor, 1910, Jour. Biol. Chem., 8, 227; Wakeman, *ibid.*, 8, 233.

EXERCISE VI.—(OPTIONAL) PICRIC ACID

1. **Dye-test.**—A woolen and a cotton yarn are left in the solution over night, and washed. The wool is colored, the cotton not. This may be applied directly to the organ-extracts, etc.

2. **Isopurpuric Acid Reaction.**—Heat the solution with KCN: blood-red color.

3. **Isolation.**—Chapter IV, Exercise III, Nos. 1 to 4.

4. **Demonstration in Urine.**—Boil the urine with HCl; extract with ether; evaporate the ethereal layer and apply the tests.

EXERCISE VII.—(OPTIONAL) NITROBENZOL

Dissolve in alcohol; reduce to anilin with zinc dust and HCl (one-half hour); make alkaline with NaOH; extract with ether, and test for anilin.

EXERCISE VIII.—(OPTIONAL) ATOPHAN (PHENYL-QUINOLIN CARBOXYLIC ACID)

Atophan urines give the following reactions (Skorczewski and John, 1911, Wien. klin. Woch., No. 49): (a) A few drops added to concentrated HCl color this bright yellow.

(b) Phosphotungstic acid gives a yellow precipitate.

(c) The addition of ammonium sulphate with ammonia gives a dark green color.

(d) The Ehrlich diazo-reaction appears after twenty-four hours.

EXERCISE IX.—(OPTIONAL) PHENOLPHTHALEIN

1. Acid solutions are colorless, but turn red with alkalis.

2. The red color disappears on heating with zinc dust.

3. **Estimation in Tablets.**—Kebler, Jour. Amer. Pharm. Assoc., 3, 1096.

QUESTIONS ON CHAPTER VII

(a) Describe a characteristic test for phenol; acetanilid; antipyrin; salicylate.

(b) How would you isolate phenol from stomach contents?

(c) How would you isolate salicylic acid from urine?

(d) How would you isolate acetanilid from a headache powder?

CHAPTER VIII

SPECIAL TESTS OF IMPORTANT ALIPHATIC DERIVATIVES

EXERCISE I.—ETHYL ALCOHOL

Most of the tests are not distinctive, but are given by other alcohols, aldehyds, esters, etc. Use (about) 1 per cent. solution for the following tests:

1. **Anstie Chromate Test.**—Add some $K_2Cr_2O_7$ solution and dilute H_2SO_4 and warm: green color and odor of aldehyd or acetic acid.

2. (Optional) **Hanzlik Contact Test** (Jour. Biol. Chem., 1912, 11, 61).—1 c.c. of the alcohol solution is placed in a test-tube; then, 0.5 c.c. of the reagent is introduced by a pipet under the alcoholic layer, without mixing: blue or light green ring at contact, becoming more intense, then fading. The reagent consists of 0.5 gm. potas. dichromate in 75 gm. concentrated sulphuric acid. This is the *most delicate test*, sensitive to 1 : 10,000.

3. **Lieben's Iodoform Test.**—Add some NaOH and iodine solution; heat gently: odor of iodoform; and precipitate of this substance may be seen consisting of microscopic hexagonal plates. The test is sensitive to 1 : 5000 and is not given by pure methyl alcohol.

4. (Optional) **Berthelot Test.**—Add a little benzoyl chlorid, shake well, let stand a few minutes, and add excess of KOH: odor of ethyl benzoate. Sensitive to 1 : 2000.

5. (Optional) **Flame Test.**—Place 1 pint of beer in a liter flask. Stopper tightly with a perforated cork bearing an upright glass tube of a bore of $\frac{1}{8}$ inch and at least 4 feet high. Heat slowly to boiling, and continue the heat until the foaming subsides. Apply a lighted match to the upper end of the tube: The alcohol vapor will ignite, most of the watery vapor being condensed in the long tube.

6. (Optional) **Isolation from Tissues, etc., and Quantitative Estimation.**—Make strongly acid with phosphoric acid and distil until all the alcohol is removed (Test 2). Filter the distillate (cotton in condensing tube). The alcohol percentage of the distillate is calculated from its specific gravity (details, Hanzlik, 1 (c)).

A permanganate method for very small quantities is described by Barendrecht, 1913, ref., Zentr. Bioch. Bioph., 14, 901. Estimation in *blood*, Abderhalden, 5, 195. Estimation of small quantities of *vapor*, Baudrexel, 1911, Zentr. Bioch. Bioph., 11, 543; Hamill, 1910, Jour. Physiol., 39, 476; Abderhalden, 5, 1046; *General Methods* of quantitative determination, Abderhalden, 2, 1.

7. (Optional) **Estimation of Alcohol in Pharmaceutic Preparations.**—See U. S. P. IX; Vanderkleed, 1909, Amer. Jour. Phar., 81, 129.

8. (Optional) **U. S. P. Purity Tests.**

9. (Optional) **Examination of alcoholic liquors**, Abderhalden, 7, 339.

EXERCISE II.—(OPTIONAL) METHYL ALCOHOL

Distinction from ethyl alcohol is especially important.

1. **Reduction Test.**—Add 1 c.c. of 1 : 1000 potassium permanganate: methyl alcohol is decolorized at once; ethyl only after twenty minutes.

2. **Formaldehyd Test** (Mulliken and Scudder).—Apply the following tests to two solutions, one containing 10 per cent. of ethyl alcohol, the other 5 per cent. of methyl and 5 per cent. of ethyl alcohol. Determine which sample is adulterated. Place 10 c.c. of the solution in a large test-tube. Heat a spiral of copper wire red hot and plunge into the solution. Repeat this five or six times. (This converts methyl alcohol into formaldehyd; the further test is for this substance.) Filter. Boil very gently until the odor of acetaldehyd disappears. Pour into a test-tube and cool. Add 1 drop of 0.5 per cent. resorcin solution; shake. Pour a portion of this liquid into a second test-tube containing concentrated sulphuric acid, held in an inclined position, so that the two liquids do not mix. Let stand three minutes and rotate slowly: A rose-red ring indicates methyl alcohol (due to formation of formaldehyd).

3. **Formic Acid Test.**—The methyl alcohol is oxidized by hydrogen peroxid into formic acid, Schmiedel, 1913; ref., Yearb. Amer. Phar. Assoc., 2, 379.

4. **Determination in Blood and Tissues.**—Nicloux, 1912 and 1913; ref., Chem. Abstr., 6, 3102; and Zentr. Bioch. Bioph., 16, 158.

OTHER TECHNICAL REFERENCES

Bukowski, 1910, Centr. Bioch., 10, 55; Deniges, 1910, Zentr. Bioch. Bioph., 10, 300; Simmonds, 1912, Amer. Jour. Phar., 85, 457; Szeberenyi, 1913, Zentr. Bioch. Bioph., 15, 635.

5. **Tests of Methyl Alcohol in Liquors.**—Vivario, 1914; ref., Zentr. Bioch. Bioph., 18, 620.

EXERCISE III.—(OPTIONAL) AMYL ALCOHOL (FUSEL OIL)

1. **Marquardt Test.**—Add a little water and 1 per cent. permanganate to red color. Let stand for a day in stoppered vessel: valerianic odor.

2. **Demonstration in Alcoholic Liquors.**—Hollaender, ref., Zentr. Bioch. Bioph., 9, 783.

EXERCISE IV.—(OPTIONAL) ACETONE

1. **Lieben's Test.**—As for alcohol (Exercise I, No. 3). In distinction from alcohol, acetone gives the test also with ammonia and ammonium iodid (Gunning).

2. **Legal's Test.**—Add a few drops of fresh sod. nitroprussid solution and make alkaline with NaOH: red color (not given by alcohol); acidulate with acetic acid: carmin color (difference from acetaldehyd, creatin, creatinin, and p-cresol).

3. **Penzoldt Indigo Test.**—Add saturated watery solution of o-nitrobenzaldehyd and NaOH: yellow, then green color; after ten minutes, blue precipitate of indigotin, soluble in chloroform. Not given by alcohol or acetaldehyd.

TECHNICAL REFERENCES

Acetone Substances (Acetone, Diacetic Acid) in *Urine and Blood*.—Abderhalden's Handb., 3, 906, 921; 5, 197, 1222; Cervello and Girgenti, 1914, Arch. exp. Path. Pharm., 75, 153; Marriott, 1913, Jour. Biol. Chem., 16, 281; in blood, 18, 508; Sammet, 1913, Zs. physiol. Chem., 83, 212; Folin, Jour. Biol. Chem., 3, 177; Folin and Denis, 1914, Jour. Biol. Chem., 18, 263 (turbidity method).

Beta-oxybutyric Acid.—Abderhalden, 3, 924, 5, 199; Shaffer and Marriott, 1913, Jour. Biol. Chem., 16, 265; Marriott, ibid., 18, 508; Kennaway, 1914, Bioch. Jour., 8, 230; Folin and Denis, 1914, Jour. Biol. Chem., 18, 263; Shaffer and Hubbard, 1916, Proc. Amer. Soc. Biol. Chem., 3, 27; Van Slyke, 1916, Proc. Soc. Exp. Biol. Med., 13, 134.

EXERCISE V.—(OPTIONAL) ETHER

The chemic tests are not characteristic. The purity tests of the U. S. P. may be applied. (An extensive discussion of these is given by Baskerville and Hamor, 1911, Jour. Ind. Eng. Chem., 3, 301, 378.)

Estimation of Ether.—Nicloux, 1906, Bioch. Centr., 6, 48. Determination in air, Kochmann and Strecker, 1912, Zentr. Bioch. Bioph., 14, 14.

EXERCISE VI.—CHLOROFORM

1. **Schwarz's Reaction.**—To a watery solution of chloroform add a trace of resorcin and a few drops of NaOH, and heat: pink color.

2. (Optional) **Lustgarten's Reaction.**—Dissolve 0.1 gm. of alpha-naphthol in strong KOH: heat to 50° C. and add a few drops of the suspected solution: blue color. Acidulate: brick-red precipitate.

3. (Optional) **Hoffmann's Reaction.**—Heat gently with alcoholic NaOH and a few drops of anilin: isonitrile odor.

4. (Optional) **Isolation.**—Distillation of the acidulated material.

5. (Optional) **Quantitative Estimation.**—Decomposition of vapors by combustion with CaO, or boiling with alcoholic KOH.

6. (Optional) **Purity Tests of U. S. P.**

TECHNICAL REFERENCES

Estimation in Air and Vapors.—Kochmann and Strecker, 1912, Zentr. Bioch. Bioph., 14, 14; Hewitt Anesthetics, 26; Mavelung, 1910, Arch. exp. Path. Pharm., 62, 414; Nicloux, 1910, Zbl. Bioch. Bioph., 10, 495.

Estimation in Blood.—Loth, 1911, Zbl. Bioch. Bioph., 12, 239.

Waller Gas Balance.—Hewitt Anesthetics, 103; Boothby and Sandiford, 1914, Jour. Pharmacol., 5, 369.

Alcohol in Chloroform.—Nicloux, 1905, Jahrb. Pharm., 66, 170.

Distinction Chloroform and Chloral.—Jona, 1911, Chem. Abstr., 6, 1337.

EXERCISE VII.—(OPTIONAL) CHLORAL HYDRATE

This gives all the reactions of chloroform.

1. To a watery solution add NaOH: odor of chloroform.
2. Nessler's Reagent gives a brick red precipitate, gradually changing to yellowish green (difference from chloroform).
3. **Isolation.**—Distillation of acidulated material.
4. **Chloral Urine.**—The chloral is excreted mainly as urochloralic acid (trichlorethyl glycuronic acid), which is decomposed by boiling with dilute acids into trichlorethyl alcohol and glycuronic acid. The urine, therefore, gives the Fehling test and polarizes to the left. Urochloralic acid is isolated by the method of Kuelz (Arch. ges. Physiol., 33, 221) or Mehring and Musculus (Gadamer, 295; Abderhalden's Handb., 3, 970).

EXERCISE VIII.—(OPTIONAL) SULPHONAL

The dry powder is decomposed by heating with:

1. Powdered wood charcoal: formation of a mercaptan (odor) and formic acid (litmus).
2. Reduced iron: mercaptan odor; residue with HCl yields H₂S.
3. KCN: mercaptan odor and KSCN (extract gives red color with ferric chlorid).
4. **Isolation.**—Proceed by Chapter IV, Exercise III, Nos. 1 to 4. Watery extracts must be filtered hot.
5. **Determination in Urine.**—Morro, 1894, Deut. Med. Woch., 34.

EXERCISE IX.—(OPTIONAL) VERONAL

1. Acidulate a saturated solution with HCl and add a few drops of Millon's reagent: white gelatinous precipitate, soluble in excess of the reagent.
2. **Isolation from Tissues or Urine.**—Proceed by Chapter IV, Exercise III, Nos. 1 to 4.

TECHNICAL REFERENCES

Isolation and Detection.—Gadamer, 458; Panzer, 1908, Bioch. Centr., 8, 167; Heiduschka, Jahrb. Pharm., 71, 463; Macadie, Chem. Abstr., 7, 1526.

EXERCISE X.—(OPTIONAL) ALDEHYD REACTIONS

1. Nessler's reagent gives a yellowish-red color, gradually changing to black, especially on heating.
2. Ammoniacal silver solution is reduced in the dark (silver mirror).
3. Fuchsin-sulphurous acid is gradually colored red.

EXERCISE XI.—(OPTIONAL) PARALDEHYD

1. This gives all the aldehyd reactions.
2. It gives the Lieben and Legal tests; see Acetone, Exercise IV.

EXERCISE XII.—FORMALDEHYD

This gives all the general aldehyd reactions. The special reactions may be divided into those which occur with weakly alkaline reaction (1, 2, and 3); strongly alkaline reaction (4), and strongly acid reaction (5 and 6). Since the stronger reagents may liberate formaldehyd from its compounds, only the first class (Nos. 1 to 3) can be used when testing for free formaldehyd in the presence of its derivatives (hexamethylenamin, etc.).

In the following tests use 1 : 50,000 solution of formaldehyd (1 drop of official liquor per liter).

S. M.—Formaldehyd, 1 : 50,000; Jorissen phloroglucin reagent; phenylhydrazin hydrochlorid, 0.5 per cent.; sod. nitroprussid, 5 per cent.

1. **Jorissen Phloroglucin Test.**—To 1 or 2 c.c. of the suspected solution add 0.5 c.c. of the reagent (phloroglucin 0.1 gm. in 10 c.c. of 10 per cent. NaOH; keeps well): pink to red color, becoming more intense, then gradually fading. The test is sensitive to 1 : 10,000,000, and may be applied directly to all body fluids, even when tinged with blood, but not to bile or undiluted blood.¹ The test may be simplified by adding a trace of dry phloroglucin to the fluid after making this distinctly alkaline with NaOH.

2. **Rimini Phenylhydrazin Test (Burnam's Test).**—To about 10 c.c. of the suspected fluid add 3 drops of 0.5 per cent. phenylhydrazin hydrochlorid; 2 drops of 5 per cent. sod. nitroprussid; and 3 drops of 10 per cent. NaOH: emerald green to deep blue color, changing to orange or red. Water alone gives a greenish-yellow color with the test, changing more rapidly to red. Formaldehyd urine may first give a purple color. The test is sensitive to 1 : 1,000,000 and may be applied directly to all body fluids except bile and whole blood (Hanzlik).

3. (Optional) **Phenylhydrazin-ferricyanid Test.**—Substitute 5 per cent. ferricyanid for the nitroprussid in the Rimini test: red color. More delicate.

4. (Optional) **Lebbin's Test.**—To about 10 c.c. of the suspected fluid add 0.5 gm. of resorcin and an equal volume of 50 per cent. NaOH; boil: red color.

5. **Liebermann's Test.**—Mix some of the formalin solution with a drop of 5 per cent. phenol and pour cautiously, without mixing, on some concentrated H_2SO_4 in test-tube: crimson zone.

6. **Hehner's Test.**—To about 5 c.c. of the solution add 1 c.c. of milk or peptone solution. Pour this on an inch of concentrated sulphuric acid containing a trace of ferric chlorid: violet zone; this test may be *applied directly to suspected milk*, by pouring this on the ferric-sulphuric acid.

7. (Optional) **Formation of Hexamethylenamin.**—This occurs when formaldehyd solution is evaporated with ammonia. It may be recognized by the precipitation reactions.

8. (Optional) **Isolation of Formaldehyd.**—Distillation of weakly acid liquid; 300 c.c. of the liquid material (or if solid, 200 gm. moistened with 100 c.c. of water) are acidulated with phosphoric acid and distilled, collecting the first 40 to 50 c.c. This is then tested either by Hehner's method or by any of the other tests.

9. (Optional) **Quantitative Estimation.**—Collins and Hanzlik, 1916, Jour. Pharmacol., 8, 130.

TECHNICAL REFERENCES

Tests.—Abderhalden's Handb., 2, 14; Dunning, 1913, Amer. Jour. Pharm., 85, 453; Hald, 1911, Arch. exp. Path. Pharm., 64, 329.

EXERCISE XIII.—HEXAMETHYLENAMIN (UROTROPIN)

Use (about) 1 : 100 solution.

1. **Bromin Precipitation.**—To the solution add bromin-water, drop by drop: orange precipitate, which redissolves until more of the reagent is added. This and the other precipitation tests are not given by free formaldehyd. They may be applied to normal urine, but not to any fluids containing proteins.

2. (Optional) **Alkaloidal Precipitants.**—Precipitates are given with mercuric chlorid, Millon's, Mayer's, Phosphomolybdic, and other alkaloidal precipitants.

S. M.—Milk; formaldehyd milk (0.1 c.c. formald. sol. per liter).

¹ Hanzlik and Collins, Arch. Int. Med., 1913, 12, 578.

3. **Liberation of Formaldehyd.**—Hexamethylenamin is decomposed by acids into formaldehyd and ammonium. It therefore gives the formaldehyd Tests 5 and 6 directly, but 1 and 2 only after treatment with acids.

(a) Render the hexamethylenamin solution freely acid with HCl and boil (or let stand in stoppered test-tube): odor of formaldehyd. To some of this solution add excess of NaOH: odor of ammonia. Use the remainder of the solution for (b).

(b) Apply the Jorissen test (1) to some of the boiled acid solution: positive.

(c) Apply the Jorissen test (1) and Liebermann's test (5) to some of the fresh hexam. solution: 1 is negative, 5 is positive.

4. **(Optional) Tests in Urine.**—Hexamethylenamin is excreted as such by the kidneys, and gives the bromin test (1) directly. In acid urines, a small quantity of formaldehyd is liberated continuously, giving the Jorissen and Rimini tests (Exercise XII, Nos. 1 and 2). Alkaline urines do not give this test, but respond to Liebermann or Hehner tests (Exercise XII, Nos. 5 and 6).

Acid hexamethylenamin urine does not usually show bacterial turbidity when kept for a day in the incubator.

5. **(Optional) Quantitative Methods.**—Falk and Sugiura, 1916, Jour. Pharm. Exp. Ther., 8, 39.

6. **(Optional) Test for Hexam. in Blood or Bile.**—Acidulate, distil, and test for formaldehyd.

TECHNICAL REFERENCES

Determination in Galenic Mixtures.—Puckner and Hilpert, 1908, Jour. Amer. Chem. Soc., 30, 1471.

EXERCISE XIV.—(OPTIONAL) FORMIC AND ACETIC ACIDS

These are the only volatile aliphatic acids of toxicologic importance. They are distinguished by their characteristic odor and taste.

1. Ferric chlorid, in neutral solution, gives a red color with both. On heating, the solution darkens and then gives a brown precipitate.

2. Mercuric chlorid, on boiling, is reduced to calomel (white precipitate) by formic acid, not by acetic.

3. Mercurous nitrate, on warming, is reduced to metallic mercury by formic acid. Acetic acid does not reduce, but on cooling concentrated solutions deposit crystalline plates of mercurous acetate, soluble on heating.

4. Silver nitrate is also reduced by formic acid, not by acetic.

5. Dry sodium acetate, heated in a test-tube with equal volumes of alcohol and concentrated sulphuric acid, gives the odor of ethyl acetate (acetic ether). Formate gives a different odor (rum) and evolution of CO.

6. **Quantitative Test for Formates in Food.**—Croner and Seligman, 1907, Bioch. Centr., 6, 306.

7. **Quantitative Estimation of Formic Acid in Urine, etc.**—(Pohl) Sollmann, 1908, Jour. Amer. Med. Assoc., 51, 821; Franzen and Greve, 1909, ref., Amer. Pharm. Assoc., 58, 355; Freyer, 1895, Chem. Ztg., No. 51, 1184; Dakin, Janney and Wakeman, 1913, Jour. Biol. Chem., 14, 341.

TECHNICAL REFERENCES

Acetic Acid.—Abderhalden's Handb., 2, 20.

EXERCISE XV.—(OPTIONAL) VALERATES

Dilute sulphuric acid liberates valeric acid, of characteristic odor.

EXERCISE XVI.—(OPTIONAL) CITRATES, TARTRATES, OXALATES, AND OTHER ORGANIC ACIDS

1. **Citrates.**—Calcium chlorid does not precipitate in the cold, but gives a white granular precipitate on boiling. Isolation from other acids: Albahary, Zentr. Bioch. Bioph., 13, 337; Tests, etc., Abderhalden's Handb., 2, 32.

2. **Tartrates.**—These give a white crystalline precipitate with potassium salts. Ammoniacal silver nitrate solution gives a metallic silver mirror on heating. Further tests, etc., Abderhalden's Handb., 2, 32.

3. **Oxalates.**—(a) To a solution of potassium oxalate add CaCl_2 : Precipitate. Add acetic acid: does not dissolve. Add dilute HCl : solution.

(b) *Isolation of Free Oxalic Acid.*—Mix the material with sand and dry on water-bath, pulverize, and extract with boiling alcohol for several hours (reflux condenser). Filter; make slightly alkaline with KOH and boil for one-half hour. Dilute with water and evaporate the alcohol. Acidulate with acetic acid and precipitate with CaCl_2 (let stand). Wash the precipitate with hot water; boil with sodium carbonate; filter; neutralize with acetic acid and precipitate the oxalate with lead. Filter; suspend the precipitate in water; and decompose with H_2S . Filter and crystallize.

(c) *Isolation of Soluble Oxalates.*—The material left from the alcoholic extraction in (2) is extracted with water, boiled, and the protein precipitated with acetic acid. The filtrate is precipitated with CaCl_2 , etc., as in (2). Details, Gadamer, 400; Tests, etc., Abderhalden's Handb., 2, 40.

4. **Lactic Acid.**—Abderhalden, 2, 28; *determination*, Wolff, 1914, Jour. Physiol., 48, 341; *in organic material*, Bellet, 1913, Zentr. Bioch. Bioph., 15, 556, 635; *in tissues and fluids*, Yoshikawa, 1913, *ibid.*, 16, 10; Meissner, 1915, Bioch. Zs., 68, 175; *in urine*, Ryffel, 1709, *ibid.*, 10, 384; *in blood*, Abderhalden, 5, 194; *in feces*, *ibid.*, 5, 387.

5. **Malic Acid.**—Abderhalden, 2, 34.

6. **Succinates.**—*Ibid.*, 2, 24.

EXERCISE XVII.—(OPTIONAL) FATTY ACIDS AND FATS

1. **Volatile Fatty Acids.**—Abderhalden's Handb., 5, 386.

2. **Butyric Acid.**—*Ibid.*, 2, 20.

3. **Oleic Acid.**—*Determination*, Polano, Zs. Geburtsh. Gyn., 65, 584.

4. **Fats.**—Abderhalden's Handb., 2, 199; 7, 184; *Determination*, Kumagawa-Suto method, *ibid.*, 5, 476; *in feces*, *ibid.*, 5, 363; Saxon, 1914, Jour. Biol. Chem., 17, 99; Laws and Bloor, 1916, Amer. Jour. Dis. Child., 11, No. 3; changes by freezing, Smith, Miller and Hawk, 1915, Jour. Biol. Chem., 21, 395; *in blood*, Abderhalden's Handb., 5, 161; Bloor, 1914, Jour. Biol. Chem., 17, 377; *in milk*, Bloor, 1914, Jour. Amer. Chem. Soc., 36, 1300; *iodin and saponification values*, U. S. P. IX.

5. **Glycerin.**—Isolation and tests, Gadamer, 388; *Reactions*, Deniges, 1900, Jahrb. Pharm., 69, 173; Ganassini, 1913, Zentr. Bioch. Bioph., 14, 772; *in blood*, Abderhalden, 5, 196; *Determination in galenicals*, Briggs, 1915, Jour. Amer. Pharm. Assoc., 4, 75; Bradts, *ibid.*, 4, 78.

6. **Acrolein.**—Qualitative, Ganassini, 1913, Zentr. Bioch. Bioph., 14, 772.

EXERCISE XVIII.—(OPTIONAL) LIPOIDS

"Lipoids" are the intracellular substances soluble in fat solvents, but exclusive of simple fats and fatty acids. They consist chiefly of lecithin and cholesterins. "Lipins" cover all substances soluble in fat solvents.

Preparation.—Abderhalden's Handb., 5, 613; *Nerve*, *ibid.*, 2, 774; *Brain*, Mathews, Physiol. Chem., 875, 15.

Phosphatids.—*Ibid.*, 2, 256; *Solubility*, *ibid.*, 3, 548; *Partition Coefficient*, *ibid.*, 3, 549; Separation of Lipins from Lipin Extracts, Rosenbloom, 1914, Soc. Exp. Biol. Med., 11, 98; *Cerebrosids*, Smith and Mair, 1911, Zentr. Bioch. Bioph., 11, 540.

Cholesterin.—Abderhalden's Handb., 2, 244; *Quantitative Estimation*, Wasker and Hueck, 1913, Arch. exp. Path. Pharm., 71, 372; 74, 416; Lifschuetz, 1913, Zentr. Bioch. Bioph., 16, 6; Schreiber, *ibid.*, 15, 788; Thaison and Hess, 1914, Bioch. Zs., 62, 89 (comparison); Weltmann, 1913, Wien. Klin. Woch., 874 (approximate colorimetric); *in blood*, Abderhalden's Handb., 5, 166; Bloor, 1916, Jour. Biol. Chem., 24, No. 3; *in erythrocytes*, *ibid.*, 5, 205; *in feces*, *ibid.*, 5, 366.

Lecithin.—Merck's Reports, 26; W. Koch, 1906, Zs. physiol. Chem., 47, 327; Koch and Woods, 1913 (lecithans), Jour. Biol. Chem., 1, No. 2; *Preparation*, Lawson and Woronzow, 1913, Arch. int. Pharmacod., 22, 394; from blood-corpuscles, Abderhalden, 5, 204; for hypodermic use, Mondolfi, Chem. Abstr., 1911, 6, 1337; *Determination and emulsification*, Schippers, 1912, Bioch. Zs., 40, 189; *in blood*, Abderhalden, 5, 166; Bloor, 1915, Jour. Biol. Chem., 22, 133.

Phytosterin.—Demonstration in animal fats, Kuehn, Bengen, and Wewerinke, 1915, *ref.*, Zentr. Bioch. Bioph., 18, 362.

EXERCISE XIX.—HYDROCYANIC ACID

1. Notice odor (which, however, may be confused with benzaldehyd or nitrobenzol).

2. **Schönbein Reaction.**—Impregnate some filter paper with freshly prepared Tincture Guaiac, let dry, then pour on some very dilute CuSO_4 ; expose this to the vapor of 1 : 1000 HCN: deep blue color (Pagenstecher, Schönbein, Preyer). Expose another paper prepared in a similar manner to the vapor of NH_3 : green color.

This test can be applied directly to suspected material, stomach washings, etc. A negative reaction definitely excludes HCN; but a positive reaction is not distinctive: the reaction depends upon the liberation of ozone by the interaction of HCN and CuSO_4 ; and ozone may be formed in other ways.

3. **Berlin-blue Reaction.**—Add to 1 : 1000 solution of HCN some FeSO_4 and Fe_2Cl_6 and a few drops of NaOH; boil, let stand a few minutes, acidulate with concentrated HCl, and heat: green to blue color, or precipitate of ferric ferrocyanid (Husemann, Ittner).

4. (Optional) **Liebig Sulphocyanid Reaction.**—Render the solution slightly alkaline with NaOH, add a little yellow ammonium sulphid, and evaporate on water-bath. Dissolve in water, acidulate with HCl and add a drop of dilute ferric chlorid: red color of ferric sulphocyanid.

5. (Optional) **Isolation of HCN.**—The material is acidulated with tartaric acid and distilled. The HCN is in the first fractions of the distillate.

The presence of sulpho-, ferro-, or ferricyanids could give rise to errors, since these may be partly decomposed in the distillation. If their presence is demonstrated (color reactions with ferric chlorid), the liquid is made alkaline, heated to 60°C . and the HCN carried over with a current of CO_2 (Jacquemin-Otto).

6. (Optional) **Determination of Small Quantities.**—Viehöver and Johns, 1915, Amer. Jour. Phar., 87, 261; in *plant tissues*, Alsberg and Black, 1916, Jour. Biol. Chem., 25, No. 1.

7. (Optional) **Estimation in Organs.**—Waller, 1910, Jour. Physiol., 40, xlvii.

8. (Optional) **Sulphocyanids.**—These give a red color with ferric salts after acidulation with hydrochloric acid. *Tests and quantitative estimation*, Abderhalden's Handb., 3, 259; in *saliva*, Autenrieth and Funk, 1912, Muench. med. Woch., 59, 2657, 2736; Gies and Kahn, 1913, Chem. Abstr., 7, 1049.

EXERCISE XX.—(OPTIONAL) CARBON DISULPHID

1. Heat a few drops with alcoholic lead acetate: black color of PbS .

2. Evaporate a few drops with alcoholic ammonia on water-bath to dryness. Formation of sulphocyanid, which gives red color with ferric chlorid.

EXERCISE XXI.—(OPTIONAL) PIPERAZIN (DIETHYLENDIAMIN)

1. **Reactions.**—Precipitation by alkaloidal precipitants; especially characteristic is a scarlet red crystalline precipitate with bismuth-potassium iodid.

2. **Demonstration in Urine.**—Add a little NaOH to precipitate earthy phosphates. Filter; render filtrate weakly acid with HCl, warm to 40°C . and add bismuth potassium iodid solution. If amorphous precipitate occurs at once, filter. The characteristic crystalline scarlet red precipitate appears after a time.

QUESTIONS ON CHAPTER VIII

1. How would you test a solution for the presence of alcohol?
 2. How would you test stomach contents for the presence of chloroform or chloral?
 3. How would you test milk for formaldehyd?
 4. How would you test hexamethylenamin urine—(a) for hexamethylenamin; (b) for free formaldehyd; (c) for bound formaldehyd?
 5. How would you test stomach contents for cyanid?
- S. M.—Tr. guaiac; HCN, 1 : 1000.

CHAPTER IX

SPECIFIC TESTS OF IMPORTANT HEAVY METALS

The ordinary tests for inorganic substances are so well covered in the usual courses of qualitative analysis that they need not be repeated. Those which are of especial medical interest are cited, mainly for convenient reference. Their special application to the urine is practically important. The substances are arranged alphabetically in each chapter.

All the exercises of this chapter are *optional*.

EXERCISE I.—ALUMINUM

1. **Reactions.**—NaOH gives a white precipitate, soluble in excess; ammonia, a white precipitate insoluble in excess.

2. **Alum in Baking Powders.**—Incinerate about 2 gm. Extract with boiling water and filter. Add to filtrate a few drops of ammonium chlorid solution: flocculent precipitate indicates alum (Off. Agric. Chem.).

3. **Isolation.**—Destroy organic matter by Fresenius-Babo. Precipitate with ammonia. Dissolve in NaOH; reprecipitate with ammonium chlorid.

Determination in Feces.—Schmidt and Hoagland, 1912; Jour. Biol. Chem., 11, 387.

Estimation in Tissues.—Gies et alias, 1916, Bioch. Bul., 5, 151.

EXERCISE II.—ANTIMONY AS TARTAR EMETIC

1. Mineral acids precipitate antimonous acid (SbO_2H_3), soluble in excess.

2. Alkalies precipitate the oxid, Sb_2O_3 , soluble in excess of KOH or NaOH, not in carbonates or ammonia.

3. Hydrogen sulphid gives a yellow color in neutral solutions, an orange precipitate in the presence of HCl.

4. **Estimation.**—Cloetta, 1911, Arch. exp. Path. Pharm., 64, 352; Brunner, 1912, Ibid., 68, 186.

EXERCISE III.—ARSENIC

1. **Reduction Test for Solid Arsenic Trioxid.**—Place powder in the bottom of difficultly fusible test-tube shown in Fig. 4. In the constricted portion place a splinter of freshly roasted wood charcoal. Heat the charcoal to redness, then the arsenic: this is

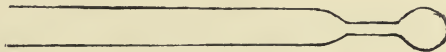


Fig. 4.—Arsenic reduction tube.

volatilized and reduced to As in passing over the carbon, and condenses in the cold parts of the tube to a black mirror. In the upper parts it is oxidized to arsenic trioxid and deposited as a white octaëdral sublimate. There is also the characteristic garlic odor.

2. **Arsenic Solutions.**—(a) Hydrogen sulphid gives lemon-yellow color or precipitate, dissolving colorless in ammonium carbonate.

(b) Acidulate with nitric acid; add silver nitrate; filter if necessary; and pour on filtrate dilute ammonia without mixing: lemon yellow zone of silver arsenite, soluble in ammonia and nitric acid.

3. **Reinsch's Test.**—This may be applied also to impure solutions. Boil a slip of thin bright copper foil (about 1 cm. square) in a test-tube with 10 c.c. of concentrated HCl: If the reagents are pure, it remains bright. Add some of the suspected liquid and boil again for one-half hour: a dark stain may denote As, Sb, Sn, Hg, Bi; no stain proves the absence of these metals.

To distinguish arsenic from other metals the foil is placed in a narrow test-tube and heated: the arsenic volatilizes and deposits in the colder parts as As or As_2O_3 .

4. **Biologic Test.**—Cultures of *Penicillium brevicaulis*, when growing on arsenic media, develop a garlic odor. The test is characteristic, being simulated only by tellurium

and selenium. Under proper conditions it is extremely sensitive (to 0.001 mg.) and applicable to impure solutions. Technic, Abderhalden's Handb., 5, 3.

5. **Marsh Test.**—See page 52. The material must be free from organic matter. Details, Gadamer, 155. *The evolution of hydrogen* is facilitated by first laying the zinc in a solution of CoCl_2 acidulated with sulphuric acid.

6. **Isolation of Arsenic from Tissues, Urine, etc.**—See page 52.

7. **Quantitative Estimation.**—Gadamer, 168; *in organs and tissues*, Joachimoglu, 1914, Arch. exp. Path. Pharm., 78, 1.

8. **Arsenic in Wall Paper, etc.**—A piece of the paper is ignited and the flame extinguished: the glowing paper has the garlic odor. Positive result shows dangerous quantity of arsenic. Smaller amounts may be demonstrated as in the tissues.

9. **Arsenic in Pharmaceutic Preparations, etc.**—See U. S. P. IX.

EXERCISE IV.—ORGANIC ARSENIC DERIVATIVES

1. **Cacodylic Acid.**—(a) *Reactions.*—This does not give the arsenic tests, except after decomposition by the Kjeldahl process. Solutions treated with zinc and sulphuric acid (or phosphorous acid in the case of urines) give the characteristic odor of cacodyl oxid.

(b) *Isolation from Urine* (Vitali).—Urine of patient receiving cacodylate. Render acid and concentrate; add equal volume of chloroform and sufficient alcohol so that the fluids mix. Then add enough water to separate the chloroform. Draw off and evaporate the chloroform. Test residue for cacodylic acid as in 1 (a).

(c) *Estimation in Urine.*—References, Merck's Report, 1910, 24, 6.

2. **Atoxyl.**—This gives the Reinsch and Marsh tests for As directly, and the tests for aromatic amido groups. The solution, when mixed with a few drops of sodium nitrite and HCl, forms a diazo compound which gives the following reactions:

(a) With alkaline phenol solution to alkaline reaction: purple color.

(b) With alpha-naphthylamin hydrochlorid: purple color.

(c) With beta-naphthylamin hydrochlorid: brick red color.

(b) and (c) are made more certain by the presence of urea.

(d) *Detection in Urine.*—Urine of patient receiving atoxyl. Apply the diazo tests as above. If the urine is deep colored it may be partly decolorized with a little boneblack.

(e) *Estimation.*—Engelhardt and Winters, 1915, Jour. Amer. Phar. Assoc., 4, 1468.

3. **Salvarsan.**—(a) *Abelin Test for Urine.*—Urines of patients receiving salvarsan or neosalvarsan give the following test, although it is doubtful whether this is specific: Acidulate about 8 c.c. of the urine with 5 or 6 drops of dilute HCl and add 3 or 4 drops of 0.5 per cent. sodium nitrite. Add a few drops of this mixture to 6 c.c. of a colorless alkaline solution of resorcin: red color in the presence of salvarsan (Muench. med. Woch., 1911, 1002 and 1566).

(b) *Determination in Tissues.*—Richter, 1911, ref., Chem. Abstr., 5, 2396.

EXERCISE V.—BISMUTH

1. The insoluble bismuth salts, when dissolved in just sufficient warm nitric acid and poured into a large excess of water, give a white precipitate, insoluble in tartaric acid.

2. Hydrogen sulphid gives a black precipitate.

EXERCISE VI.—CHROMIUM

1. Chromates give a yellow precipitate with barium or lead salts; a red precipitate with silver or mercurous.

2. To about 5 c.c. of hydrogen peroxid solution in a test-tube add a little sulphuric acid, a thin layer of ether, and a trace of the chromate: blue color of the ether (hyperchromic acid).

3. **Isolation.**—Destruction of organic matter, see page 52. Evaporation to dryness; fusion with saltpeter; solution in water.

EXERCISE VII.—COPPER

1. **Reactions.**—Ammonia gives a deep blue color; ferrocyanid a red brown precipitate; metallic iron acquires a coating of metallic copper.

2. **Test in Stomach Contents.**—Acidulate with HCl and place in platinum crucible with a piece of metallic zinc: copper deposit on platinum.

3. **Isolation from Tissues, etc.**—Destruction of organic matter, see page 52. Precipitation with hydrogen sulphid; solution in nitric acid; evaporation to dryness; solution in water.

EXERCISE VIII.—IRON

The medicinal iron preparations are either salts of iron, or the iron is a firmly bound constituent of the molecule. The first class (inorganic irons) give the ordinary iron reaction; the latter (organic or masked iron) do not.

1. **Reactions of Ferrous Salts.**—Hydrogen sulphid gives a greenish-black precipitate of FeS , soluble in mineral acids; alkalis a white precipitate turning blue, green, and brown; potassium ferrocyanid a white precipitate; ferricyanid a blue precipitate; sulphocyanid no color.

2. **Reactions of Ferric Salts.**—Hydrogen sulphid as for ferrous. Tannin gives a blue or greenish-black color; alkalis a brown precipitate; ferrocyanid a blue precipitate; ferricyanid a brown color; sulphocyanid a blood-red color, bleached by mercuric chlorid, not by alcohol. The red ferric sulphocyanid is extracted by ether.

3. **Reactions of Salts with Organic Acids.**—"Scale-salts," such as ferric citrate or ferric ammonium citrate or tartrate, do not precipitate with hydrogen sulphid, ammonia, or ferro- or ferricyanid. They are precipitated by NaOH (ferric hydroxid). After acidulation, they are also precipitated by ferrocyanid.

4. **Distinction of Ionic (Inorganic) and Non-ionic (Organic or Masked) Iron.**—(a) *MacCallum's Reaction.*—This is the most delicate: a drop of fresh $\frac{1}{2}$ per cent. hematoxylin solution gives a blue-black color with inorganic iron, but not with organic. The test is best applied to the dry substance or concentrated solution. Confirm that the following preparations are correctly classed:

Inorganic: Ferric sulphate.
Scale salt of iron.
Iron albuminate.

Organic: Dried blood.
Egg-yolk.
Iron somatose.

(b) The action of dilute hydrochloric acid liberates the inorganic iron from some of the masked compounds, but not from others. To demonstrate this, add a little 5 per cent. hydrochloric acid and a drop of potassium ferrocyanid to ovoferin and to egg-yolk, and boil: the first gives the Prussian blue reaction, the second not.

Lay some alcohol hardened sections of spleen in the ferrocyanid, and others in the acid-ferrocyanid mixture. Spleen contains loosely bound organic iron (ferratin) and therefore colors in the acid mixture, but not in the plain ferrocyanid.

TECHNICAL REFERENCES

Abderhalden's *Handb.*, 5, 1101; *Estimation of traces*, Jahn, 1911, *Zs. physiol. Chem.*, 78, 308; in presence of organic matter, Salkowski, *Ibid.*, 43, 142.

EXERCISE IX.—LEAD

1. **Lead Acetate.**—This gives a yellow precipitate with dichromates or iodids; a white precipitate with sodium hydroxid, chlorids, or bromids; a black precipitate with hydrogen sulphid. The subacetate is also precipitated by acids.

2. **Lead Carbonate (White Lead).**—(a) Heated with sodium carbonate by blow-pipe, it fuses to the ductile metal and a yellow deposit. The metal may be dissolved in nitric acid and tested as lead acetate.

(b) It is blackened by hydrogen sulphid.

3. **Isolation from Foods, Tissues, Feces, Urine, etc.**—Destruction of organic matter, see page 52. Solution of the PbCl_2 in hot water; neutralization by ammonia to weakly acid reaction; precipitation with hydrogen sulphid; solution in nitric acid.

TECHNICAL REFERENCES

Erlenmeyer, 1913, *Bioch. Zs.*, 56, 330; Friedmann, 1914, *Zs. physiol. Chem.*, 92, 46; *urine*, Ohio State Board of Industrial Health Hazards, pp. 387 and 388.

4. **Detection in Drinking-water.**—Black precipitate with hydrogen sulphid shows dangerous amount. Smaller quantities may be demonstrated by adding sodium phosphate to 1 to 10 liters of the water, standing twenty-four hours, decanting, dissolving the precipitate in dilute nitric acid, evaporating the excess of acid, and precipitating with hydrogen sulphid.

5. **Excess of Lead in Vessels, Solder, etc.**—Boil for one-half hour with 4 per cent. acetic acid; evaporate the solution; precipitate with hydrogen sulphid.

6. **Colorimetric Estimation of Traces.**—Siegfried and Pozzi, 1914, *Bioch. Zs.*, 61, 149.

EXERCISE X.—MANGANESE

1. **Reactions of Permanganates.**—The color of the aqueous solution is discharged by oxidizable substances (NaNO_2). In alkaline reaction there is also a brown precipitate. KOH changes the color of permanganate to green, with evolution of oxygen.

2. **Isolation of Manganese.**—Destruction of organic matter, see page 73. Precipitation with ammonium sulphid (flesh colored in pure solutions); fusion with sodium carbonate and nitrate: green fusion mass if manganese is present.

EXERCISE XI.—MERCURY

1. **Reduction by Copper** (Applicable to Impure Solutions).—Reinsch test, see page 50: gray deposit. Dry and rub lightly with filter paper: silver color. Heat in narrow test-tube: stain disappears and is deposited on tube as gray mercury mirror; magnification shows Hg globules. Place tube in stoppered flask containing a little iodine. In a few hours red mercuric iodide is formed (or the original copper foil with its deposit may be laid on a glass slide, next to a small piece of iodine, and covered with a watch-glass).

2. **Reduction by Tin.**—To a solution of mercuric chloride add a fresh solution of stannous chloride: white precipitate of HgCl . More of the reagent, with heat, gives a gray precipitate of Hg.

3. **Klein's Test.**—To the mercurial solution add a little KI, a drop of ammonium chloride, and then NaOH, drop by drop: brown or yellow color or precipitate (NH_4I). This test is very delicate and may be made still more so as a contact method, adding the NaOH containing NH_4Cl without mixing.

4. **Isolation of Mercury from Tissues or Urine.**—Destruction of organic matter, see page 50. Precipitation by H_2S . Solution in HCl with KClO_3 ; evaporation at 50° to 60° C. Solution of the residue in water is suitable for the preceding tests.

Mercuric chloride, iodide, or cyanide may be extracted directly from the dried material by ether.

5. **Determination of Minute Traces.**—Strzyzowski, ref., Chem. Abstr., 7, 805.

6. **Quantitative Estimation in Urine or Tissues.**—As sulphid or electrolytic.

TECHNICAL REFERENCES

Buchtala, 1913, Zs. Physiol. Chem., 83, 212 and 249. Gadamer, 213; *In urine*, Kobert, Intox., 2, 335; Abelin, Zentr. Bioch. Bioph., 13, 829; Siebert, Ibid., 10, 434; Klotz, 1914, Zs. Physiol. Chem., 92, 286; Perelstein and Abelin, 1915, Muench. med. Woch., Aug. 31 (highly delicate test by precipitation with basic lead acetate); *destruction of organic matter*, E. Salkowski, Bioch. Zs., 61, 27.

7. **Quantitative Estimation in Bichloride Tablets.**—Chapin, 1914, Amer. Jour. Pharm., 86, 1; La Wall, 1914, Jour. Amer. Phar. Assoc., 3, 50; Kebler, Ibid., 3, 1087, 1091.

8. **Calomel.**—(a) Lime-water gives a black mixture.

(b) KI solution gives a yellow, green, gray, or black color.

(c) *Estimation:* Grantham, 1915, Jour. Amer. Pharm. Assoc., 4, 441; *in Tablets*, Kebler, 1914, Jour. Amer. Pharm. Assoc., 3, 1089.

EXERCISE XII.—PHOSPHORUS

1. **Scherer's Preliminary Test.**—Place some phosphorus water in a small bottle; stopper it loosely and between the cork and the neck of the bottle suspend two pieces of filter paper, the one impregnated with Silver Nitrate, the other with Lead Acetate. If the silver paper is blackened and the lead paper not, the presence of Phosphorus is rendered probable. (If both are blackened, this indicates H_2S .)

2. **Luminous Ring Test.**—See page 50.

3. **Fresenius-Neubauer.**—The material, in a flask, is acidulated with sulphuric acid and distilled at 60° to 70° C. in a current of CO_2 . The vapors are passed through 3 per cent. silver nitrate: phosphorus causes a precipitate.

A hydrogen apparatus is arranged as in the Marsh test and the hydrogen is ignited. The silver precipitate is introduced into the flask. The flame is colored green if phosphorus is present.

4. **Determination of White Phosphorus:** Engelhardt and Winters, 1915, Jour. Amer. Pharm. Assoc., 4, 451; **in Matches:** Phelps, 1914, Hyg. Lab. Bul. No. 96.

EXERCISE XIII.—SILVER

1. **Reactions of Silver Nitrate.**— NaCl gives a white curdy precipitate, insoluble in nitric acid, soluble in ammonia. This solution, heated with formaldehyd, deposits a metallic mirror.

2. **Isolation from Tissues, etc.**—Destruction of organic matter, see page 52. The precipitated AgCl is collected. Any remaining in solution is precipitated by hydrogen sulphid, dissolved in nitric acid, evaporated to dryness, and precipitated with HCl. The united AgCl is dissolved in ammonia and tested with hydrogen sulphid, aldehyds, and blowpipe fusion with KCN (silver granule).

3. **Determination of Traces.**—Malatesta, 1915, ref., Zentr. Bioch. Bioph., 18, 85.

4. **Determination of Ag in Protein Compounds.**—Incineration (2 gm.); solution of residue in warm, dilute nitric acid. Titration with sulphocyanid.

5. **Determination in Colloid Silver Preparations.**—Dankwort, 1915, ref., Zentr. Bioch. Bioph., 18, 252.

EXERCISE XIV.—ZINC

1. **Reactions of Soluble Zinc Salts.**—(a) White precipitate with ammonium sulphid, insoluble in acetic acid, soluble in HCl. (b) White precipitate with ferrocyanid, soluble in KOH.

2. **Zinc Oxid.**—Turns lemon yellow on heating. Easily soluble in dilute acid, giving the reactions of soluble zinc salts. Also soluble in NaOH.

3. **Isolation.**—Destruction of organic matter, see page 52. Addition of excess of sodium acetate; precipitation (hot) with hydrogen sulphid; solution in nitric acid; conversion into oxid by incineration; solution in dilute acetic acid.

EXERCISE XV.—ANALYSIS OF RARE ELEMENTS

Abderhalden, 8, 269.

EXERCISE XVI.—TESTS FOR HEAVY METALS

(See U. S. P. IX for "Limit test.")

QUESTIONS ON CHAPTER IX

1. How would you test a powder suspected of being arsenic trioxid?
2. Give an outline of the Marsh test.
3. Give an outline of the Reinsch test.
4. How would you test stomach contents for copper?
5. Describe a test for ferric salts.
6. Describe a test for ferrous salts.
7. Describe a test for differentiating organic iron.
8. How would you test a tablet for the presence of mercuric chlorid?
9. How would you determine whether a white powder is calomel?
10. How would you recognize phosphorus in stomach contents?

CHAPTER X

SPECIAL REACTIONS OF EARTHY AND ALKALI METALS

The cations are arranged alphabetically. All the exercises of this chapter are optional.

EXERCISE I.—AMMONIUM

Heated with alkalis, ammonium salts evolve ammonia vapors having the characteristic odor and bluing litmus.

TECHNICAL REFERENCES

Estimation, Abderhalden's Handb., 3, 765; *in urine*, Ibid., 5, 285; rapid, 7, 719; *in blood*, Ibid., 5, 156; rapid, 7, 724; *in feces*, Ibid., 5, 357; Rosenbloom, 1913, clinical for *urine*, Jour. Amer. Med. Assoc., 61, 87; substitute for *Nessler's Reagent*, S. S. Graves, 1915, Jour. Amer. Chem. Soc.

EXERCISE II.—BARIUM

1. **Reactions.**—(a) White precipitate with sulphates (even calcium sulphate solution), insoluble in dilute acid. (b) Dichromate gives a yellow precipitate, insoluble in acetic acid (difference from lead). (c) The nitrate and chlorid color the Bunsen flame green.

2. **Differences from Strontium.**—The latter is not precipitated at once by calcium sulphate. It is not precipitated by dichromate. It colors the flame red.

3. **Isolation of Barium.**—Destruction of organic matter, see page 52; the insoluble residue is saved. In the filtrate the greater part of the acid is neutralized, and the Ba precipitated with sulphuric acid. The precipitate is added to the original insoluble residue; dried and incinerated; oxidized with nitric acid; again heated to redness, and fused with potas. sodium carbonate. The mass is extracted and the precipitated barium carbonate dissolved in dilute HCl.

EXERCISE III.—CALCIUM

Ammonium oxalate gives a white precipitate, insoluble in acetic acid, soluble in HCl. White precipitates are also given by sodium carbonate or phosphate; both precipitates are soluble in acid. The sulphate precipitate is insoluble in acids.

TECHNICAL REFERENCES

Estimation in Urines, Organic Fluids, etc.: Abderhalden's Handb., 5, 293; Gutmann, 1914, Zentr. Bioch. Bioph., 16, 359; Goy, 1913, Ibid., 16, 359; Bell, 1912 (clinical), Bioch. Jour., 6, 205; Stransky, 1914, Arch. exp. Path. Pharm., 78, 122; v. d. Heide, 1914, Bioch. Zs., 65, 363; H. Lyman, 1915 (rapid method), Jour. Biol. Chem., 21, 551; in *blood*, Halverson and Bergeim, 1916, Proc. Am. Soc. Biol. Chem., 3, 22; microcolorimetric, Howland, Haessler, and Marriott, 1916, *ibid.*, 3, 18.

EXERCISE IV.—LITHIUM

1. **Reactions.**—Precipitate on warming with sodium phosphate, but not with carbonate or sulphate. It colors the flame crimson, with characteristic spectrum.

2. **Detection in Urine.**—Evaporate and incinerate. Extract with dilute HCl; evaporate; extract with alcohol; evaporate; spectrum test. *Quantitative Estimation*, Murmann, 1910; Chem. Abstr., 5, 2607.

EXERCISE V.—MAGNESIUM

Sodium phosphate with ammonium chlorid and ammonia give a white crystalline precipitate. NaOH or carbonate cause precipitation. No precipitate is given by bicarbonate, sulphate, or oxalate. *Estimation in Urine*, Abderhalden's Handb., 5, 293; in *tissues*, Stransky, 1914, Arch. exp. Path. Pharm., 78, 122.

EXERCISE VI.—POTASSIUM

1. **Reactions.**—Lilac tint to colorless flame. Tartaric acid gives white crystalline precipitate. Platinic chlorid gives yellow crystalline precipitate of potassioplatic chlorid. Sodiodobaltic nitrite solution gives yellow precipitate.

2. **Quantitative Determination in Urine.**—Abderhalden's Handb., 5, 292; 5, 1113; H. J. Hamburger, 1915 (traces), Bioch. Zs., 71, 415.

EXERCISE VII.—SODIUM

Yellow tinge to flame.

EXERCISE VIII.—STRONTIUM

Crimson flame. Sulphates give white precipitate, soluble in strong acids. Distinction from barium, see Exercise II. Isolation, as for barium.

QUESTIONS ON CHAPTER X

1. How would you determine whether a cough mixture contains an ammonium salt?

2. How would you determine whether a cathartic salt is magnesium sulphate, sodium sulphate, or sodium phosphate?

CHAPTER XI

CAUSTIC MINERAL ACIDS AND ALKALIES; PEROXIDS

All the exercises are optional.

EXERCISE I.—FREE MINERAL ACIDS

These need only be considered if the reaction is strongly acid to litmus. In the case of organs an aqueous or alcoholic extract is used.

1. **Demonstration of Free Mineral Acid.**—(a) *Methyl-violet Test.*—A 1 per cent. methyl-violet solution is diluted with water to a light violet color. Mineral acids change the color to blue, green, and yellow. With oxalic acid the yellow is indistinct.

(b) *Iodin Test.*—A very dilute solution of ferric acetate, mixed with KI and starch solution, is gradually colored blue when notable quantities of free mineral acid are present.

2. **Distinction of the Acid Radicle.**—This may be done as in Chapter XII.

3. **Demonstration of Free HCl.**—This is important, since Cl ions are present in all tissues. In the absence of other volatile mineral acids this may be done by heating the sample on the water-bath with a drop of methyl-violet: the green color would gradually return to violet if the acidity was due to HCl.

EXERCISE II.—CAUSTIC ALKALIES

These need only be considered if the reaction is freely alkaline to litmus. Their quantity is determined volumetrically in an aqueous extract, directly, and after preceding precipitation with barium chlorid (to determine the share of the carbonates). The cathion is identified as in Chapter X.

EXERCISE III.—HYDROGEN PEROXID

1. It evolves oxygen on contact with a crystal of permanganate.

2. Dilute solutions do not liberate iodine from KI and starch, but do so on adding a crystal of ferrous sulphate.

3. The solution is rendered acid with sulphuric acid, a drop of very dilute dichromate is added, and the mixture shaken at once with ether: the latter is colored blue.

QUESTIONS ON CHAPTER XI

How would you determine whether the strongly acid reaction of a bloody vomitus is due to mineral acid?

CHAPTER XII

SPECIAL REACTIONS OF INORGANIC ACID RADICALS

The anions are arranged alphabetically. All the exercises are optional.

EXERCISE I.—BICARBONATES

These give the reactions of the carbonates, but do not precipitate earthy metals from their salts until boiled (boiling converts them into carbonates).

EXERCISE II.—BORATES AND BORIC ACID

1. **Reactions.**—(a) *Flame Test.*—Over a little boric acid (or sodium borate with a drop of sulphuric acid) in an evaporating dish pour some alcohol and ignite: green flame.

(b) *Turneric Test.*—Turneric (curcuma) paper, immersed in boric acid (or borate acidulated with HCl) and dried, turns brownish red. Ammonia changes this to bluish black.

2. **Determination in Urine, Tissues, Food, etc.**—(a) *Rough Method.*—Add $\frac{1}{8}$ of concentrated HCl to the suspected fluid (e. g., milk with 1.5 gm. per liter) or extract, and apply the turmeric test 1 (b).

(b) *Exact Method.*—About 25 gm. of the material (more in the case of urine) is made alkaline with lime-water, evaporated to dryness, incinerated, extracted with 15 c.c. of water and sufficient HCl to make it freely acid. Apply turmeric test 1 (b).

3. **Quantitative Estimation.**—Off. Agr. Chem.; F. C. Cook, 1916, Jour. Agr. Res., 5, 877.

EXERCISE III.—BROMIDS

1. **Reactions.**—(a) Silver nitrate gives a yellowish-white precipitate (AgBr), insoluble in dilute nitric acid, soluble in ammonia.

(b) Chlorin-water in solutions acidulated with sulphuric acid liberates bromin, which dissolves in chloroform with yellow color.

2. **Detection in Urine.**—Evaporate and incinerate at low heat. Extract with water and test by 1 (b). If large quantities are present, this test may be applied directly to the urine.

3. **Quantitative Estimation in Urine, Blood, and Tissues.**—Bernouilli, 1913, Arch. Exp. Path. Pharm., 73, 365; Larsson, 1913, Bioch. Zs., 49, 479; Bogdandy, 1913, Zbl. Bioch. Bioph., 15, 59; Autenrieth and Funk, 1912, Muench. med. Woch., 59, 2657, 2736 (colorimetric); Takeda, 1911, Arch. internat. Pharmacodyn., 21, 203; Bermann, 1910, Ther. Mon., 183 (urine); Wyss, 1906 and 1908, Arch. exp. Path. Pharm., 45, 266; 49, 186; Halogens in lipoids, Capenberg, 1912, Chem. Abstr., 6, 1014. *In medicines*, Leech, 1915, Rep. Chem. Lab., A. M. A., 8, 54.

4. **Isolation of Free Bromin from Stomach Contents, etc.**—Passage of current of air through material in a flask, catching the bromin in water, and shaking this with chloroform.

EXERCISE IV.—CARBONATES

1. White precipitates, soluble in dilute nitric acid, are given with the salts of Ca, Ba, Mg, Pb., etc.

2. Acids liberate CO₂ gas, which precipitates lime-water, but not calcium chlorid.

EXERCISE V.—CARBONIC ACID

1. **Reactions.**—See Exercise IV, 2.

2. **Excess of CO₂ in Air.**—Large excess is demonstrated by the extinction of a candle flame. Quantitative estimation by Pettenkofer's barium method (Gadamer, 49).

TECHNICAL REFERENCES

Estimation, Abderhalden's Handb., 3, 600; *in blood*, Ibid., 5, 157; *minute quantities*, Tashiro, 1913, Amer. Jour. Physiol., 32, 107, 137; *alveolar air*, Y. Henderson and Russell, 1912, Ibid., 29, 436; Comparison of methods of obtaining, Boothby and Peabody, 1914, Arch. Int. Med., 13, 497. *Indicator method*, Haas, 1916, Sci., 44, 105.

Carbon.—Graphic demonstration in lung, E. F. Hirsch, 1916, Jour. Amer. Med. Assoc., 66, 950.

Gas Analysis.—Abderhalden's Handb., 3, 555; 5, 1027 (Zunz); Haldane, 1898, Jour. Physiol., 22, 465; Abderhalden, 3, 683; Table of *Volume Reduction*, Abderhalden, 3, 599; *micro-analysis*, Ibid., 3, 658.

Preparation of Gases.—Ibid., 1, 215, 230; *Air analysis*, Heinz, 2, 452.

Blood-gases.—Abderhalden's Handb., 3, 664; Heinz, 2, 437; Tigerstedt, 2.1, 1; Brodie, 1910, Jour. Physiol., 39, 391; Brodie and Cullis, 1908 (saline), Ibid., 36, 405; *Absorption and Tension*, Fahr, 1910, Jour. Physiol., 43, Abderhalden, 3, 699; *in circulating blood*, Ibid., 3, 703.

Intestinal Gases.—Abderhalden, 5, 415.

Oxygen Estimation.—Ibid., 3, 622. *Determination of oxygen content of water*, Winkler-Hyman, Amer. Jour. Physiol., 40, 241, 1916.

EXERCISE VI.—CHLORATES

1. **Reactions.**—(a) Evolution of Cl gas on heating dry chlorate with concentrated HCl.

(b) Added to sulphuric acid and indigo solution, there is no change; but decolorization occurs when a trace of sulphurous acid is added.

(c) Chlorates do not precipitate silver nitrate, but do so on the addition of sodium bisulphite.

2. **Isolation of Potassium Chlorate from Stomach Contents or Tissues.**—Extract by dialysis; concentrate by evaporation; precipitate with alcohol; crystallize from water.

3. **Detection in Urine.**—Test 1 (b) may be applied directly to the urine (Rabuteau).

4. **Isolation from Urine.**—Decolorize with lead subacetate. Remove lead from filtrate with hydrogen sulphid; evaporate and crystallize.

5. **Quantitative Estimation in Urine.**—Hildebrandt, 1906, ref., Bioch. Centr., 5, 831.

EXERCISE VII.—CHLORIDS

Silver nitrate gives a white curdy precipitate, insoluble in nitric acid, soluble in ammonia.

Lead salts also give a white precipitate, soluble in boiling water, insoluble in ammonia.

TECHNICAL REFERENCES

Microchemic, Abderhalden's Handb., 5, 1131; *Estimation in Urine*, Ibid., 5, 291; Symes, Jour. Physiol., 32, 221; simplified, McLean and Selling, 1914, Jour. Amer. Med. Assoc., 62, 1081; in presence of SCN, Cormimboeuf, 1912, Chem. Abstr., 7, 39. *In Blood*, etc., Abderhalden, 5, 207; Gazzetti, 1913, Zbl. Bioch. Bioph., 15, 791; McLean and Van Slyke, 1915, Jour. Biol. Chem., 21, 223, 361, 509. *Cl ions in blood*, Abderhalden, 7, 727. *Centrifugation method*, Sueyoshi, 1916, ref., Jour. Amer. Med. Assoc., 66, 929.

EXERCISE VIII.—CHROMATES

(See page 74.)

EXERCISE IX.—FLUORIDS

1. **Reactions.**—(a) Dry NaF, moistened with concentrated sulphuric acid, evolves HF, which etches glass.

(b) Solutions give a white precipitate with Ca or Ba salts.

2. **Determination in Blood.**—Abderhalden, 5, 159.

3. **Detection in Foods.**—*Detection of Fluorids* (used as food preservatives): 150 c.c. of the sample (or in the case of solid foods, the aqueous extract) are brought to boiling, and mixed with 5 c.c. of 10 per cent. K_2SO_4 and 10 c.c. of 10 per cent. barium acetate. The precipitate is allowed to settle, collected on a small filter, washed, and incinerated in a platinum crucible. A glass plate is coated with wax and some marks scratched through the wax with a pointed stick. The contents of the crucible are moistened with concentrated H_2SO_4 , and covered with the waxed plate, the edges of the crucible being firmly embedded in the wax. The glass is now covered with a cooling device and the crucible is heated for an hour as high as is possible without melting the wax. The glass plate is now removed and cleaned (by steam): a distinct etching proves that fluorin was present.

EXERCISE X.—GLYCEROPHOSPHATES

1. They do not give a precipitate with ammonium molybdate in the cold, but do so on heating.

2. The dry salts, when strongly heated, evolve inflammable vapors and leave a residue of pyrophosphate.

3. A saturated aqueous solution of calcium glycerophosphate deposits white iridescent scales of anhydrous calcium glycerophosphate on boiling.

EXERCISE XI.—HYPOCHLORITES

These evolve Cl gas (odor) on the addition of acids.

EXERCISE XII.—HYPOPHOSPHITES

1. Solutions acidulated with sulphuric acid and mixed with silver nitrate give a white precipitate, changing rapidly to brown or black by reduction to metallic silver.

2. Solutions heated with copper sulphate produce a red-brown precipitate of cuprous oxid.

3. Ca or Ba salts are not precipitated.

EXERCISE XIII.—IODIDS

1. **Reactions.**—(a) Silver nitrate gives a yellow precipitate, insoluble in dilute nitric acid, practically insoluble in ammonia.

(b) Lead acetate gives a yellow precipitate, soluble on heating.

(c) Mercuric chlorid gives a red precipitate, soluble in excess of either reagent.

(d) To an iodid solution add a little sodium nitrite and dilute sulphuric acid: liberation of iodine, with yellow or brown color, dissolving in chloroform or carbon disulphid with violet color, and turning starch solution blue.

(e) Chlorine-water also liberates iodine.

2. **Qualitative Test in Urine or Saliva.**—To about 5 c.c. of the urine or saliva add a few drops of concentrated sulphuric acid and of 1 per cent. sodium nitrite solution. Shake out with chloroform: violet color of the chloroform.

3. **Quantitative Estimation of Iodids in Tissues, etc.**—Hanzlik, 1910, Jour. Biol. Chem., 7, 259; in blood, Abderhalden's Handb., 5, 160; in lipoids, Cappenberg, 1912, Chem. Abstr., 6, 1014. *Electrolytic determination*, Krauss, 1916, Jour. Biol. Chem., 24, No. 3.

4. **Estimation of Organic Iodine in Thyroid.**—Hunter, 1910, Jour. Biol. Chem., 7, 321; Kendall, 1911, Proc. Soc. Exp. Biol. and Med., 8, 120; 1915, Jour. Biol. Chem., 19, 251; Bernier and Perron, 1911, Zentr. Bioch. Bioph., 12, 57; F. C. Koch, 1913, Jour. Biol. Chem., 14, 106.

5. **Estimation in Presence of Bromids.**—Bray and MacKay, 1910, Jour. Amer. Chem. Soc., 32, 1193; Kendall, 1912, Chem. Abstr., 6, 2867.

EXERCISE XIV.—IODINE

1. **Reactions.**—(a) Odor, color, and violet vapor on heating.

(b) Blue color with starch solution, discharged by thiosulphate.

(c) Dissolves in chloroform or carbon disulphid with violet color.

(d) Forms iodoform with NaOH and alcohol.

2. **Detection in Stomach Contents.**—(a) Brown color of protein material, discharged by thiosulphate or ammonia.

(b) Violet color of chloroform extract.

3. **Stains.**—As in 2 (a).

EXERCISE XV.—NITRATES

1. **Reactions.**—(a) To a 5 per cent. solution of potassium nitrate add an equal volume of concentrated sulphuric acid; cool, and drop in a crystal of ferrous sulphate: dark brown color of ferric sulphate around the crystal.

(b) Mix a nitrate solution with solution of ferrous sulphate and add a layer of concentrated sulphuric acid: brown ring.

(c) Add a drop of diphenylamin solution and a layer of concentrated sulphuric acid: deep blue contact ring.

(d) Free nitric acid (or nitrate with HCl) discharges the color of indigo on heating.

2. **Detection of Nitrate in Stomach Contents or Urine.**—Evaporate the alkaline watery extract or urine to dryness; dissolve in a little water and test by 1 (a).

3. **Quantitative Estimation in Urine.**—Caron, ref., Chem. Abstr., 6, 2442.

4. **Potassium Nitrate in Meat.**—Off. Agr. Chem.

EXERCISE XVI.—NITRIC ACID

1. **Reactions.**—See Nitrates, Exercise XV.

2. **Stains.**—Yellow color of organic tissues, deepened to orange by ammonia.

Isolation from Stomach Contents.—Extract rapidly with alcohol; neutralize with calcium carbonate, filter, and evaporate the alcohol.

EXERCISE XVII.—NITRITES

Acid solutions liberate iodine from KI and decolorize permanganate.

Estimation of Nitrous Oxide.—Abderhalden's Handb., 3, 655.

Analysis of Nitrous Oxide.—Boothby and Sandiford, 1915, Amer. Jour. Physiol., 37, 371.

Nitroglycerin.—Estimation of small quantities, Scoville, 1911, Amer. Jour. Pharm., 83, 359; in tablets, Keblor, 1914, Jour. Amer. Pharm. Assoc., 3, 1094.

EXERCISE XVIII.—PERMANGANATES

(See page 76.)

EXERCISE XIX.—PHOSPHATES (ORTHO-)

1. Magnesia mixture gives a white crystalline precipitate.
2. Silver nitrate gives a yellow precipitate, soluble in ammonia and nitric acid.
3. Ammonium molybdate and heat gives a yellow precipitate.

Estimation in Urine.—Abderhalden's Handb., 5, 290; **total phosphorus in tissues**, etc., A. E. Taylor and Miller, 1914, Jour. Biol. Chem., 18, 215; Neumann, 1902, Zs. physiol. Chem., 37, 129; Chapin and Powick, 1915, Jour. Biol. Chem., 20, No. 2; Forbes, Beegle, and Wussow, Ohio Agr. Exp. Sta. Tech. Bul. 8. Microcolorimetric method for serum, Howland, Haessler, and Marriott, Proc. Amer. Soc. Biol. Chem., 3, 18, 1916.

EXERCISE XX.—SILICATE (SODIUM)

Acids produce a gelatinous precipitate of silicic acid.

Determination in Urine.—Salkowski, Zs. physiol. Chem., 43, 142.

EXERCISE XXI.—SULPHATES

Barium chlorid or lead acetate give white precipitates, insoluble in dilute acids.

Estimation.—Abderhalden's Handb., 3, 794; 5, 288, 307; Johnston and Adams, 1911, Jour. Amer. Chem. Soc., 33, 829; *volumetric*, North, 1914, Amer. Jour. Pharm., 86, 249; **total S. in Urine**, Denis, 1910, Jour. Biol. Chem., 8, 401; *volumetric*, Raiziss and Dubin, 1914, Jour. Biol. Chem., 18, 297; *Conjugated, after drugs*, Abderhalden, 3, 947, 955.

EXERCISE XXII.—SULPHURIC ACID

Isolation.—Extraction with alcohol; neutralization with NaOH; evaporation; solution in water.

EXERCISE XXIII.—SULPHIDS

1. **Reactions.**—They blacken lead acetate. Dilute acids liberate H_2S (odor).
2. **Detection of H_2S in Air.**—(a) Blackening of lead acetate paper. (b) Aspirate air through dilute ammonia containing a few drops of dilute nitroprussid: violet color.
3. **Quantitative Estimation:** Abderhalden's Handb., 3, 657; in Air: Lehmann; ref., Gadamer, 51.

EXERCISE XXIV.—SULPHITES

1. **Reactions.**—(a) Acids liberate sulphur dioxide (odor).
(b) Solutions blacken mercurous nitrate.
(c) Added to Zn and HCl, they develop H_2S .
2. **Detection of Sulphur Dioxide in Air.**—(a) Odor.
(b) Paper impregnated with potassium iodate and starch is colored blue.
(c) The air is aspirated through water. This gives the sulphate reactions after addition of chlorin-water.
3. **Detection of Sulphite in Meat.**—(a) Place on starch-iodate paper and moisten with dilute sulphuric acid: immediate deep blue color (late light blue is insignificant).
(b) 5 to 25 gm. are subjected to test 1 (c). The sample is placed in a 200-c.c. Erlenmeyer flask, and diluted with water if necessary. Some zinc and hydrochloric acid are now introduced, and the flask tightly stoppered, fixing a strip of lead acetate paper with the stopper. H_2S will be generated by the reduction of the sulphites, and blacken the paper. A negative result shows the absence of sulphites, but a blackening could also be due to sulphids. In this case it is necessary to distil the acidulated sample in a current of CO_2 (which may be generated directly in the flask by the addition of $NaHCO_3$). The distillate is received in a standardized solution of iodine, which is then titrated with sodium thiosulphate (also Gadamer, 53).
4. **Quantitative Estimation.**—Off. Agr. Chem.

EXERCISE XXV.—THIOSULPHATES (HYPOSULPHITES)

Mineral acids liberate sulphur dioxide and precipitate sulphur. They give a white precipitate with lead acetate, turning black on heating.

QUESTIONS ON CHAPTER XII

1. How would you test a solution for a carbonate and a bicarbonate?
2. How would you test an antiseptic solution for borate?
3. How would you determine whether an epilepsy mixture contains bromid?
4. How would you determine whether a tablet contains potassium chlorate?
5. How would you confirm that a patient is taking iodid?
6. How would you test for free iodine in a vomitus?

CHAPTER XIII

FLAVORS

The mouth should be rinsed after tasting each solution.

EXERCISE I.—SWEETENING AGENTS

Determine the sweetening power of the following drugs as compared with 1 per cent. cane-sugar. Start with the strengths given below and dilute, each time with equal quantities of water, continuing until the taste is less sweet than that of saccharose. Then try two dilutions between this and the preceding. Note any qualitative difference in taste.

Each pair of students determine the saccharin; the others will be assigned:

1. Sodium saccharin, 0.01 per cent.
2. Glycerin, 10 per cent.
3. Lactose, 10 per cent.
4. Glucose, 10 per cent.
5. Levulose, 10 per cent.

QUESTION

Tabulate the results in multiples of cane-sugar (*e. g.*, saccharin = 300 × cane).

EXERCISE II.—DILUTION

Compare the taste of the following: (*a*) undiluted; (*b*) diluted with 10 volumes of water. Taste the weaker solutions first.

- | | |
|---------------------------------------|-----------------------------|
| 1. Magnesium sulphate, 20 per cent. | } Character of taste. |
| 2. KBr (or KI), 5 per cent. | |
| 3. Sodium salicylate, 10 per cent. | Mawkish. |
| 4. Chloral, ¹ 10 per cent. | Acrid. |
| 5. Quinin bisulphate, 1 per cent. | Bitter. |
| 6. Saccharin, 0.1 per cent. | Sweet and bitter. |

QUESTIONS

(*a*) Tabulate the results, indicating whether the dilution is markedly, moderately, or scarcely effective in disguising the taste.

(*b*) For what classes of substances would dilution be effective?

(*c*) For what classes of substances would it be ineffective?

(*d*) What qualitative change is there on diluting the saccharin?

¹ Taste the strong solution cautiously.

EXERCISE III.—COMPARISON OF WATER AND MILK

Compare the taste of solutions 1 to 5 of Exercise II, diluted with 10 volumes of (a) water, (b) milk.

QUESTIONS

(a) Tabulate the results, indicating whether milk is markedly more effective than water.

(b) For what classes of substances would milk be especially indicated?

(c) For what classes of substances would it be superfluous?

EXERCISE IV.—COMPARISON OF WATER AND ACACIA

As in Exercise III, using 5 per cent. acacia instead of milk.

QUESTIONS

Analogous to Exercise III.

EXERCISE V (OPTIONAL).—COMPARISON OF WATER AND AROMATIC WATER

As in Exercise III, using peppermint-water instead of milk.

QUESTIONS

Analogous to Exercise III.

EXERCISE VI (OPTIONAL).—HOT AND COLD SOLUTIONS

As in Exercise III, comparing the diluted solutions cold and hot.

QUESTIONS

Analogous to Exercise III.

EXERCISE VII.—COMPARISON OF WATER AND SYRUP

As in Exercise III, using syrup instead of milk.

QUESTIONS

Analogous to Exercise III.

EXERCISE VIII (OPTIONAL).—CONCENTRATED AND DILUTED SYRUP

Compare the taste of solutions 1 to 5 of Exercise II diluted with 10 volumes of (a) syrup; (b) diluted syrup (1 : 10).

QUESTIONS

(a) Tabulate the results, indicating whether concentrated syrup is markedly more efficient than diluted syrup.

(b) For which classes of substances would dilution of the syrup be permissible?

(c) For which not?

EXERCISE IX.—COMPARISON OF SYRUP AND ACID SYRUP

As in Exercise VIII, comparing dilution with (a) simple syrup, (b) citric acid, 1 per cent. in syrup.

QUESTIONS

Analogous to Exercise VIII.

EXERCISE X (OPTIONAL).—SYRUP AND SYR. AURANTII CORT.

As in Exercise IX, using orange syrup instead of the citric acid.

QUESTIONS

Analogous to Exercise IX.

EXERCISE XI (OPTIONAL).—SYRUP AND TOLU SYRUP

As in Exercise IX, using Syr. Tolu.

QUESTIONS

Analogous to Exercise IX.

EXERCISE XII.—SYRUP AND GLYCYRRHIZA

As in Exercise IX, using Syr. Glycyrrh.

QUESTIONS

Analogous to Exercise IX.

EXERCISE XIII.—SYRUP AND ELIXIR

As in Exercise IX, using elixir.

QUESTIONS

Analogous to Exercise IX.

EXERCISE XIV (OPTIONAL).—SYRUP AND COMP. TINCT. GENTIAN

As in Exercise IX, using the Tincture.

QUESTIONS

Analogous to Exercise IX.

EXERCISE XV.—SYRUP AND CO. TR. CARDAMOMI

As in Exercise IX, using the Tincture.

QUESTIONS

Analogous to Exercise IX.

EXERCISE XVI.—SYRUP AND SYR. ERIODICTYON

Analogous to Exercise IX.

EXERCISE XVII.—QUININ

Compare the taste of the following in the order given:

1. Equinin (quinin ethylcarbonate).
2. Quinin adsorbed by Fuller's earth.
3. Quinin tannate.
4. Quinin alkaloid.
5. Quinin sulphate.

QUESTION

Arrange results in order of taste.

EXERCISE XVIII.—FATTY OILS

Compare the taste of cod-liver oil in the following:

1. Pure.
2. With addition of 0.4 per cent. peppermint oil.
3. With addition of 0.4 per cent. lemon oil.
4. In 50 per cent. emulsion, unflavored.

QUESTIONS

Record the efficiency in correcting the oily taste.

EXERCISE XIX.—INSIPID POWDERS

Compare the chalky taste in the following:

1. Pure chalk.
2. Chalk, 1; milk-sugar, 1.
3. Chalk, 1; cane-sugar, 1.
4. Chalk, 1; sugar, 0.5; cacao, 0.5.
5. Chalk, 1; sugar, 0.8; cinnamon, 0.2.

QUESTION

Which is the most effective flavor?

EXERCISE XX.—TASTE OF CATHARTIC SALTS

Compare the taste of the following in 5 per cent. solutions:

1. Magnesium sulphate.
2. Sodium sulphate.
3. Sodium phosphate.
4. Sodium-potassium tartrate.
5. Sodium citrate.

QUESTIONS

- (a) Record your results as to degree of disagreeable taste.
- (b) Which of the salines would be easiest to take?
- (c) Which would be the most difficult?

GENERAL QUESTIONS ON CHAPTER XIII

Which flavors would be suitable and which unsuitable for:

- (a) Saline taste (Magn. sulph., KBr, KI)?
- (b) Mawkish or alkaline taste (salicylate or bicarbonate)?
- (c) Acrid taste (ammonium chlorid or carbonate, chloral)?
- (d) Bitter taste (quinin, strychnin, etc.)?
- (e) Oily taste?
- (f) Chalky taste?

CHAPTER XIV

(DEMONSTRATION) COLORS; (OPTIONAL) DETECTION OF COLORS IN FOODS, ETC.

(DEMONSTRATION) COLORS

Tabulate the colors produced by the addition of the following colors to:

(a) *Water*; (b) *sod. bicarb.*; (c) *1 per cent. HCl*; (d) *10 per cent. suspension of chalk*.

1. Liq. Carmini,	0.3	per cent.
	1	"
	3	"
2. Tr. Cardam. Co.,	25	"
	50	"
	75	"
3. Tr. Personis,	0.3	"
	1.5	"
	5	"
4. Fld. Ext. Glycyrrh.,	5	"
	15	"
	25	"
5. Tr. Caramel, N. F.,	0.5	"
	1.5	"
	5	"
6. Tr. Hydrastis,	0.3	"
	1	"
	3	"
7. Tr. Curcumæ,	0.3	"
	1	"
	3	"
8. Methylene-blue,	0.01	"
	0.1	"
9. Chalk with Carmin,	1 : 500	(dry).

(OPTIONAL) DETECTION OF COLORS AND PRESERVATIVES IN FOODS, ETC.

The following table of commonly used preservatives and colors may serve as a guide to the examiner:

Wines and Grape-juice.—Cochineal; Coal-tar Dyes; Salicylic Acid; Sulphites.

Beer, Cider, Ginger Ale.—Caramel; Salicylic Acid.

Liquors and Vinegar.—Caramel.

Canned Vegetables, Catsups, and Other Sauces, Pickles.—Copper; Saccharin; Sulphite; Coal-tar Colors (tomatoes); Salicylic and Benzoic Acid; Borate.

Butter.—Coloring; Borax.

Milk and Cream.—Coloring; Formaldehyd; Borax.

Jams and Jellies.—Coloring, Coal-tar and Cochineal; Salicylic and Benzoic Acid; Saccharin.

Meats.—Coal-tar Colors; Sulphite; Borax; Fluorid.

Syrups.—Saccharin.

Color Standards.—Army and Ring, 1916, Jour. Ind. Eng. Chem., 8, 309.

EXERCISE I.—COAL-TAR COLORS

Dyeing Test.—Some pure white wool (nun's veiling) is cleaned by boiling for a short time with very dilute KOH, thoroughly washed, dried, and cut into pieces about 3 by 10 cm. These may be preserved in stoppered bottles.

If the suspected material is liquid, about 100 c.c. are taken. If it is solid or semi-solid, 5 to 25 gm. are diluted with water to 100 c.c. The liquid is acidulated with 2 to 4 c.c. of 10 per cent. HCl. A strip of the wool is added and the mixture boiled for five to fifteen minutes.

If the wool is not appreciably dyed in this time, no coal-tar color is present. If it is dyed, it is washed in cold water; warmed for a few minutes with very dilute HCl; and again washed thoroughly. It is then placed in a 2 per cent. ammonia solution, warming if necessary. When the cloth is nearly or quite decolorized it is taken out of the liquid, and this is diluted to 50 c.c., rendered moderately acid with HCl, and another slip of the cloth is added. It is then warmed in the water-bath. Natural colors will not give any appreciable stain in this second dyeing; whereas coal-tar colors and some lichens will give a well-marked color to the wool.

EXERCISE II.—RED COLORS

1. **NaOH Test.**—The origin of red colors may be discovered by the addition of NaOH; this causes a change to green if the pigment is that of fruits; to blue or purple if it is of other vegetable origin. Anilin dyes are not changed. For other tests, see La Wall, 1905.

2. **Cochineal.**—The foods, etc., are first extracted with water if necessary. The filtered solution is acidulated with HCl and extracted with amyl alcohol; this is colored yellow to orange if cochineal is present. The amyl alcohol layer is drawn off and washed three times with a little water. On adding a very dilute uranium acetate solution, drop by drop, to the amyl alcohol a characteristic emerald-green color appears if cochineal is present.

EXERCISE III.—YELLOW COLORS

1. **Turmeric (Curcuma).**—(a) To some of the 1 per cent. tincture add a drop of NaOH (reddish-brown color); then an excess of dilute HCl: yellow color is restored.

(b) Dip some paper which has been dyed with curcuma (Turmeric Paper) into 5 per cent. boric acid: orange color. Touch it at one place with dilute HCl and dry: deeper red. Moisten with ammonia: deep blue. (This serves also as a test for boric acid.)

In applying this test for the discovery of turmeric in solids, these are first extracted with alcohol.

2. **Artificial Coloring Matter in Milk (Leach's Method).**—Heat 150 c.c. of the milk in a porcelain capsule and add enough acetic acid (about 5 c.c.) to curdle. Stir and heat to near boiling. Gather the curd in one mass on the stirring rod or strain if necessary. The artificial color will be found in the curd.

This is pressed as dry as possible, and macerated for several hours (over night) with 50 c.c. of ether in a small, tightly corked flask. The ethereal solution is treated according to (a), the curd by (b).

(a) **Annatto.**—This is contained in the ether extract which is decanted and evaporated. The residue is made alkaline with dilute NaOH, warmed, and passed through a wetted filter. The fat is washed from the filter by a stream of water and the paper is dried. If annatto is present the paper has an orange color. A drop of stannous chlorid solution changes this to pink.

(b) If the curd, after the extraction with ether, is of a pure white color, no artificial dye is present. If it is yellowish or orange this indicates anilin orange; if it is brown, caramel is suspected. Proceed by (c).

(c) **Anilin Orange.**—A lump of the curd is shaken in a test-tube with strong HCl: it turns pink at once.

(d) **Caramel.**—If the curd is brown, and anilin orange is absent, the presence of caramel may be assumed.

(e) **Turmeric.**—This may be tested for directly in the milk.

3. **Artificial Coloring Matter in Butter.**—(a) **Annatto and Saffron.**—5 gm. of the suspected butter are dissolved in 50 c.c. of ether in a white tube and shaken vigorously with 15 c.c. of very dilute KOH (which must remain alkaline after separating). After standing a few hours the aqueous layer is drawn off (without filtering), evaporated to dryness, and moistened with concentrated sulphuric acid. Annatto gives a blue or violet blue, changing quickly to green, and finally to brown. Saffron does not give the intermediate green color.

(b) **Turmeric** is shown by extracting the melted butter with alcohol and applying the ordinary test.

EXERCISE IV.—DETECTION OF CARAMEL IN LIQUORS, ETC.

1. On shaking the sample the foam has a brown color if a considerable proportion of caramel is present.

2. Shake some of the fluid with about one-tenth its volume of Fuller's earth for two or three minutes. Filter, returning the first portions of the filtrate until it runs clear. If the color of this filtrate is markedly lighter than that of the original fluid this indicates the presence of caramel. It is always well to make comparative tests with a sample of known purity.

3. *Identification in Flavoring Extracts.*—Lichthardt, 1916, Jour. Amer. Phar. Assoc., 5, 294.

QUESTIONS

- (a) What agents and what proportions are suitable for coloring alkaline liquids pink, red, brown, yellow, blue?
- (b) Ditto for acid liquids?
- (c) Ditto for neutral liquids?
- (d) Is it advisable to color suspensions?
- (e) Write a formula for tooth powder consisting of chalk and colored pink.

CHAPTER XV

CHEMIC STUDY OF THE EXCRETION OF DRUGS IN MAN

Explanatory.—Drugs are excreted mainly by the urine and feces; to a lesser extent by the saliva, milk, and other secretions. They may also be demonstrated in effusions; a few pass into the cerebrospinal fluid, etc.

The following experiments relate mainly to the urine, and involve practice in the application of the tests and the mapping out of the course of the normal excretion.

The urinary excretion is generally parallel to the absorption, so that it also gives a fair idea of the absorption of the drugs. Readily absorbable drugs usually begin to appear in the urine within one-quarter to one-half hour, reach their maximum concentration in two to four hours, and then diminish gradually. Many drugs are temporarily stored in the body, so that traces continue to be excreted for days, weeks, or even months.

Assignment of Experiments.—The exercises should be divided among the class so that each student takes one of the drugs, collects the urine and saliva in fractions as directed, tests each of these fractions for the drug, and by the relative intensity of the reactions maps out a curve of the excretion. These results will be presented to the next meeting of the class.

The fractions showing the strongest reaction should be furnished to the instructor, who will distribute them to the remaining members of the class for practice in the tests. Each student will, therefore, study his own urines in detail, and also perform all the reactions on the most typical urines of the other students. It is also useful to have urines of pathologic cases, especially nephritis, for comparison.

Collection of Urine.—The experiments are assigned and the drugs (average doses) are given out at the previous laboratory meeting. Just before breakfast, on the morning preceding the laboratory exercise, the subject collects a sample of urine, empties the bladder, and takes the drug. The urine is collected separately at the end of the following periods after taking the drug: one-quarter hour; one-half hour; one hour; two hours; four hours; six hours; ten hours; sixteen hours; twenty-four hours. Bottles will be provided by the instructor. If the saliva is to be tested the mouth must be rinsed thoroughly after taking the drug (or this may be taken in a cap-

sule), and a small sample of saliva (5 c.c.) is collected at the end of each of these periods.

If the drug is to be applied to the skin it should be painted or rubbed on the inner side of the arm or thigh.

EXERCISE I, A.—EXCRETION OF IODID AFTER ORAL ADMINISTRATION

Test the urine *and saliva*, according to Chapter XII, Exercise XIII, No. 2, after taking one of the following drugs:

- (1) *Potassium Iodid*, 0.3 gm. in water.
- (2) *Syr. Hydriodic Acid*, 5 c.c. in water.
- (3) *Strontium Iodid*, 0.3 gm. in water.
- (4) *Syr. Ferrous Iodid*, 1 c.c. in water.
- (5) *Iodalbin* (iodin-blood protein compound), 5-grain capsule.
- (6) *Iodipin* (10 per cent. iodized sesame oil), 5 c.c. in milk.
- (7) *Sajodin* (calcium mono-iodobehenate), 1 gm. in milk.

EXERCISE I, B.—EXCRETION OF IODID AFTER DERMAL ADMINISTRATION

Test the urine *and saliva* according to Chapter XII, Exercise XIII, No. 2.

(8) *KI Ointment*.—Rub about 1 gm. of 10 per cent. KI ointment (U. S. P. VIII) into the skin (prolonged friction).

(9) *Iodin Tincture*.—Paint a square inch of the skin with Tr. Iodin.

EXERCISE II.—LIBERATION OF IODIN FROM KI BY NITRITES OF THE SALIVA

(Each pair of students should try this experiment.) Mix equal parts of 1 per cent. KI and 1 per cent. H_2SO_4 , add a little starch paste, place in three test-tubes, and add to:

(a) Saliva; (b) boiled saliva; (c) water. *a* and *b* both turn blue, while *c* remains unchanged. Since the reaction is not destroyed by boiling, it cannot be due to ferments. (It is caused by the presence of *nitrites* in the saliva; the depth of color varies greatly in different individuals.)

EXERCISE III, A.—EXCRETION OF SALICYL AFTER ORAL ADMINISTRATION

Test urines according to Chapter VII, Exercise IV, No. 3.

1. *Sodium Salicylate*, 1 gm. in water.
2. *Acetyl-salicylic Acid* (aspirin), 0.3 gm. capsule.
3. *Methyl Salicylate* (oil of wintergreen or birch), 10 drops in capsules.
4. *Phenyl Salicylate* (salol), 0.3 gm. powder.
5. *Salicin*, 1 gm. powder. (Salicin splits into dextrose and saligenin. The latter oxidizes into salicylic aldehyd and salicylic acid.)

EXERCISE III, B.—EXCRETION OF SALICYL AFTER DERMAL APPLICATION

Test urine according to Chapter VII, Exercise IV, No. 3. Paint the skin with one of the following:

6. *Sodium Salicylate*, 5 c.c. of saturated alcoholic solution.
7. *Methyl Salicylate*, 2 c.c. of 50 per cent. in olive oil.
8. *Spirosal* (monoglycol salicylate), 2 c.c. of 50 per cent. in oil.
9. *Mesotan* (methyl oxymethyl salicylate), 2 c.c. of 50 per cent. in oil.

S. M.—Weighed drugs.

EXERCISE IV.—(OPTIONAL) CONVERSION OF BENZOIC INTO HIPPURIC ACID

(See Dakin, 1910, Jour. Biol. Chem., 7, 103.)

EXERCISE V.—EXCRETION OF HEXAMETHYLENAMIN AND FORMALDEHYD IN URINE AND SALIVA

Test the urine *and saliva* according to Chapter VIII, Exercise XIII, No. 4.

Take hexamethylenamin, 0.5 gm. in water.

EXERCISE VI, A.—CONVERSION OF ORGANIC ACIDS INTO CARBONATES

The acid radicles of organic salts are largely oxidized in the body to carbonates. The acidity of the urine is thereby diminished; or, with large doses, it may become actually alkaline. Test the reaction of the urines to litmus.

1. *Sodium Acetate*, 10 gm. in water.
2. *Sodium Citrate*, 10 gm. in water.

EXERCISE VI, B.—(OPTIONAL) MEASUREMENT OF HYDROGEN ION CONCENTRATION

In considering reaction (acidity or alkalinity) it is necessary to differentiate between *total acidity or alkalinity* (the entire H or OH available for neutralizing); and *actual acidity or alkalinity* (the free or dissociated H⁺ and OH⁻ ions). The *potential acidity or alkalinity* (*reserve alkali*) corresponds to the difference between the two, *i. e.*, to the combined H or OH.

The *total acidity or alkalinity* is measured by the ordinary titration methods; the *actual reaction*, expressed as H⁺ ions, may be determined by an electrometric method, by certain indicators, or by the velocity of certain chemic reactions.

TECHNICAL REFERENCES

Total Acidity of Urine.—Abderhalden's Handb., 5, 283.

Hydrogen Ion Concentration.—Michaelis, Wasserstoffionen Konzentration, Berlin, 1914; Abderhalden's Handb., 1, 534; 5, 317, 500, 1095; Tigerstedt, 1.2, 186; Palmer and Henderson, 1913, Arch. Int. Med., 12, 153; Walpole, 1913 (gas-electrode), Bioch. Jour., 7, 410; (litmus), *ibid.*, 7, 260; (indicator chart), *ibid.*, 5, 207; Dreser, 1910 (indicators of effective alkali), Arch. internat. Pharmacod., 20, 431; McClendon, 1915 (H electrodes), Amer. Jour. Physiol., 38, 180 (direct reading potentiometer), *ibid.*, 186; Crozier and Harrison, 1915, Surg. Gyn. Obst., Dec., 722; J. H. Long, 1916, Jour. Amer. Chem. Soc., 38, 936.

Preparation of Solutions.—Abderhalden, 3, 1337.

Alkalinity of Blood.—Abderhalden, 5, 200; Peabody, 1914, Arch. Int. Med., 14, 236; Heinz, 1, 389; Levy, Rowntree, and Marriott, 1915 (dialysis method), Arch. Int. Med., 16, 389.

EXERCISE VII, A.—EXCRETION OF METHYLENE-BLUE (METHYLTHIONIN HYDROCHLORID)

Experiment 1.—Take 0.15 gm. of methylene-blue in capsule.

The urine has a blue or green color after thirty to fifty minutes. (Decolorizing under the action of bacteria.)

(a) Boil with a few drops of concentrated HCl: the color becomes pinkish red; neutralize with NaOH: returns to green.

(b) Add a few drops of NaOH, boil, and add a few drops of 1 per cent. glucose¹ solution: the color disappears, but reappears on shaking.

(Detection of chromogens in urine, Fleig, 1909, Chem. Abstr., 3, 552.)

¹ The urine often contains enough reducing substance to decolorize on heating, even without the addition of glucose. This may be tried.

Experiment 2.—Take 65 c.c. of 1.5 : 1000 solution of methylene-blue (= 0.1 gm.), previously shaken with 3 gm. of animal charcoal. The urine is not colored. Explain.

EXERCISE VII, B.—(OPTIONAL) PHENOLSULPHONEPHTHALEIN EXCRETION TEST

The rate of excretion of this substance is used as a test for renal efficiency (Rowntree and Geraghty, 1910, Jour. Pharmacol., 1, 579; 2, 393); 1 c.c. of a solution containing 6 mg. of the phthalein is injected deep into the lumbar muscle. The patient is given 300 to 400 c.c. of water about one-half hour before the drug. The urine is collected at intervals. A drop of 25 per cent. NaOH causes a deep red color.

To estimate the excretion quantitatively, NaOH is added to each sample until the color reaches its maximum. It is then diluted to 1 liter, filtered, and compared in a colorimeter with a standard solution (3 mg. per liter).

EXERCISE VIII, A.—EXCRETION OF WATER

The experiment extends over four days.

On the first day the bladder is emptied before breakfast. At breakfast the usual amount of fluid is taken (measured), and the urine collected and measured every hour for four hours.

On the second day the same routine is followed, but an additional 500 c.c. of water is taken at breakfast.

On the third day, as on the first day, with an additional 500 c.c. of milk.

On the fourth day, as on the first day, with an additional 500 c.c. of water and 1 gm. of theobromin-sodium salicylate.

QUESTIONS

1. What proportion of the additional water is excreted by urine within the four hours (a) with water; (b) with milk?
2. How soon does the additional excretion start, when does it reach its maximum, and when is it completed (a) with water, (b) with milk?
3. Which is the more efficient diuretic? Why?
4. How does theobromin affect the excretion?

EXERCISE VIII, B.—(OPTIONAL) EXCRETION OF SALT

The experiment extends over four days, with the diet as uniform as practical, especially in regard to salt. The total urine of each twenty-four hours is collected, and the percentage and total quantity of chlorid is determined. An extra 10 gm. of salt, dissolved in water, is taken at the beginning of the second day.

TECHNICAL REFERENCES

Tests of Kidney Function.—R. Fitz, 1914, Amer. Jour. Med. Sci., 148, 330; Mosenthal, 1916, Jour. Amer. Med. Assoc., 67, 933; Chace and Myers, 1916, *ibid.*, 67, 929; Myers, Fine and Lough, 1916, Arch. Int. Med., 17, 570; *Fluorescein*, Strauss, 1913, Berl. klin. Woch., 2226; *Urea index*, McLean, 1916, Jour. Amer. Med. Assoc., 66, 415; Jour. Exp. Med., 16, 733; Addis and Watanabe, 1916, Jour. Biol. Chem., 24, No. 3; *Lactose and Iodid Test*, Schlager and Takayasu, 1911, Deut. Arch. Klin. Med., 101, 333; *Nephritic Test-meal*, Hedinger and Schlager, 1914, Deut. Arch. Klin. Med., 114, 120; Mosenthal, 1915, Arch. Int. Med., 16, 733.

Tests of Liver Function.—Chesney, Marshall, and Rowntree, 1914, Jour. Amer. Med. Assoc., 63, 1533; Rowntree, Marshall, and Chesney, 1914, Trans. Assoc. Amer. Phys., 29, 586; Whipple and co-workers, 1913, J. H. H. Bul. 24, 207, 269, 343, 359; Sisson, 1914, Arch. Int. Med., 14, 804; Krumbhaar, 1914, N. Y. Med. Jour., Oct. 10; Jour. Exp. Med., 13, 136; Kahn and Johnston, 1915, N. Y. Med. Jour., Oct. 23.

Urobilin and Urobilinogen.—Lenhartz, 312; Wilbur and Addis, 1913, Trans. Assoc. Amer. Phys., 28, 617; Abderhalden, 3, 852, 856; 5, 315; Ville, 1915, Zentrbl. Bioch. Bioph., 18, 578; *Urochrome and other pigments*, *ibid.*, 3, 857; 2, 736.

EXERCISE IX.—EXCRETION OF ACETANILID DERIVATIVES

Test urines by the Indophenol Reaction, Chapter VII, Exercise II, No. 5.

1. **Acetanilid.**—0.2 gm. as powder.
2. **Acetphenetidin (Phenacetin).**—0.3 gm. as powder.

EXERCISE X.—EXCRETION OF ANTIPYRIN

Take 0.3 gm. in water and test urines according to Chapter VII, Exercise III, No. 4.

EXERCISE XI.—SANTONIN URINE

Take 0.05 gm. of santonin as powder, and test urines according to Chapter VI, Exercise II, Nos. 4 and 5, *a, b, d*.

EXERCISE XII.—EXCRETION OF EMODIN CATHARTICS

Test urines for chrysophanic acid, according to Chapter VI, Exercise II, No. 5, *d*. Also note time of cathartic effect; number and character of stools; griping, etc.

1. **F. E. Rhubarb.**—1 c.c.
2. **F. E. Senna.**—2 c.c.
3. **F. E. Cascara.**—1 c.c.

EXERCISE XIII.—EXCRETION OF QUININ

Test urine for alkaloid: acidulate with dilute sulphuric acid and add a drop of mercuric potassium iodid: precipitate disappears on heating, reappears on cooling.

1. **Quinin Sulphate.**—0.2 gm. capsule.
2. **Quinin Alkaloid.**—0.2 gm. capsule.
3. **Quinin Tannate.**—0.2 gm. capsule.
4. **Quinin Ethyl-carbonate (Euquinin).**—0.3 gm. powder.

EXERCISE XIV.—(OPTIONAL) COPAIBA URINE

Take 1 gm. of copaiba, and test the urines as follows:

(a) Add concentrated HCl: red color, becoming violet on heating. The spectroscope shows bands in the blue, green, and orange (Quincke, 1883). The reaction is not produced by all samples of the drug.

(b) Add ammonia: light brown or bluish fluorescence.

(c) Boil: precipitates; add alcohol: dissolves.

(d) Test for sugar: the result is often positive (due to glycuronic acid).

EXERCISE XV.—FORM OF ADMINISTRATION ON ABSORPTION OF WATER-SOLUBLE DRUGS

Take KI, 0.3 gm., in the following forms, and determine its urinary excretion according to Chapter XII, Exercise XIII, No. 2.

1. **Solution.**
2. **Powder.**
3. **Capsules.**
4. **Pills.**
5. **Salol-coated Pills.**¹
6. **Glutoid Capsules.**

¹ Amer. Pharmaceut. Assoc., 57, 94.

EXERCISE XVI.—FORM OF ADMINISTRATION OF INSOLUBLE ESTERS

Take phenyl salicylate, 0.3 gm., in the following forms, and determine its urinary excretion according to Chapter VII, Exercise IV, No. 3.

1. Powder.
2. Powder, with 5 parts of chalk.
3. Capsule.
4. Pill.

EXERCISE XVII.—(OPTIONAL) VEHICLE ON ABSORPTION

Take KI, 0.3 gm., in the following vehicles, and determine its urinary excretion according to Chapter XII, Exercise XIII, No. 2.

1. Dissolved in glass of water.
2. Dissolved in glass of milk.
3. Dissolved in 1 ounce of simple syrup.
4. Dissolved in 1 ounce of thick starch paste.
5. Dissolved in 1 ounce of 50 per cent. alcohol.

EXERCISE XVIII.—(OPTIONAL) STATE OF DIGESTIVE CANAL ON ABSORPTION

Take KI, 0.3 gm., in water, under the following conditions, and determine its urinary excretion according to Chapter XII, Exercise XIII, No. 2.

1. One hour before breakfast.
2. Just after breakfast.
3. One hour after breakfast.
4. Two hours after breakfast.
5. Three hours after breakfast.

QUESTIONS ON CHAPTER XV

1. State for each of the drugs used:
 - (a) When the excretion begins.
 - (b) When it reaches its maximum.
 - (c) When it begins to decline.
 - (d) When it is reduced to traces.
 - (e) When it is completed.
2. In the exercises in which several combinations of a drug were used, arrange these in the order of the rapidity of their excretion.
3. Explain why certain combinations are excreted more slowly.
4. With water-soluble drugs, arrange the forms of administration in the order of absorption, and explain the reasons for the differences.
5. Same as to insoluble esters.
6. What influence has the vehicle on absorption? Explain.
7. How does food influence absorption? Explain.
8. How could the urine be rendered alkaline without disturbing the reaction of the stomach? Explain.

CHAPTER XVI

CHEMIC ANTIDOTES

Explanatory.—One of the first objects in treating a case of poisoning is to render the poison insoluble, thereby delaying its absorption. The agent which is used for this purpose must itself be almost harmless, so that

it can be given in unlimited quantity. With this restriction any precipitant may be used. (It is useful to remember that these precipitants are generally employed as tests for the substance.) The subject is also simplified by the fact that the same chemic antidotes are used for all alkaloids.

EXERCISE I.—ANTIDOTES FOR ALKALOIDS

1. **Tannin.**—(a) To some $\frac{1}{10}$ per cent. solution of Strychnin Sulphate add a little infusion of tea: large precipitate. Add to half of this some alcohol, to the other half some dilute HCl: the precipitates dissolve.

(b) Repeat with $\frac{1}{10}$ per cent. Morphin Sulphate: only a slight precipitate.

(c) Repeat (a) with coffee infusion: only a slight precipitate.

Tannin is an efficient precipitant of some alkaloids, but not of others. Coffee is less efficient than tea. The precipitates dissolve in alcohol and in dilute acids.

2. **Iodin.**—To some saturated aqueous Quinin Sulphate add some solution of iodine in KI: large precipitate. Add some alcohol: the precipitate dissolves.

3. **Permanganate.**—To some quinin solution add solution of KMnO_4 : brown precipitate. Add alcohol: no solution.

The reactions 2 and 3 apply to all alkaloids, so that these reagents may be considered universal alkaloidal antidotes.

EXERCISE II.—ANTIDOTES FOR METALS

1. **Tannin.**—(a) Add some tea to Lead Acetate: large precipitate. Add to half of this some alcohol: no solution; to the other add dilute HCl: the tannate is decomposed and lead chlorid is precipitated.

(b) Repeat (a) with HgCl_2 : very little precipitate.

(c) Repeat (a) and (b) with coffee: results similar to tea.

Some metals are precipitated by tannin, others not. The precipitates are insoluble in alcohol, somewhat soluble in dilute acids.

Coffee-tannin is also effective, but less than tea.

2. **Proteins.**—Mix some HgCl_2 and albumin solutions: large precipitate. Practically all metals are precipitated by proteins.

EXERCISE III.—SPECIAL ANTIDOTES

1. **Barium and Sulphates.**—To some barium chlorid solution add Na_2SO_4 solution: white precipitate.

2. **Oxalates and Calcium.**—To a solution of potassium oxalate add some $\text{Ca}(\text{OH})_2$: precipitate.

3. **Phosphorus and Copper.**—Drop a small piece of phosphorus into a dilute solution of CuSO_4 : the phosphorus is soon covered with a film of metallic copper.

EXERCISE IV.—(DEMONSTRATION) BULK OF ACID AND ALKALI REQUIRED FOR NEUTRALIZATION

1. **Neutralization of Sulphuric Acid.**—Place about $\frac{1}{2}$ ounce of concentrated sulphuric acid in each of three large beakers; add to (a) Sodium Bicarbonate; (b) Magnesium Oxid; (c) Sodium Hydroxid (10 per cent.) until neutral to litmus.

S. M.—Strych. Sulph., $\frac{1}{10}$ per cent.; Morphin Sulph., $\frac{1}{10}$ per cent.; infusion of tea; infusion of coffee; egg-white solution; phosphorus in small pieces.

2. **Neutralization of Sodium Hydroxid.**—Place 1 ounce of 10 per cent. NaOH in each of two beakers; add to (a) Dilute Acetic Acid; to (b) Dilute Hydrochloric Acid until neutral to litmus.

QUESTIONS ON CHAPTER XVI

1. Tabulate the chemic antidotes for alkaloids, metals, lead, b̄rium, oxalates, phosphorus.
2. State your observations; explain the chemic changes.
3. Does the administration of the chemic antidotes suffice for the treatment of poisoning? Why?
4. Which is most effective as a precipitant, tea or coffee?
5. Should alcohol be administered when the chemic antidotes for alkaloidal poisons are employed? Why?
6. Which would be the best alkali to use against poisoning by acids, and vice versa? Why?

CHAPTER XVII

ADSORPTION BY COLLOIDS

Explanatory.—Fine solid particles have the property of condensing many dissolved substances on their surface, and thus removing them from solution. This effect increases with the surface, and, therefore, with the fineness of the particles. It is especially striking in colloid solutions, in which the particles are ultramicroscopic, transitional between solution and solid. This adsorption may be utilized to delay local action and absorption and as antidote in alkaloidal poisoning. However, this use is limited, since the dissolved matter is eventually given up in the intestines by simple solution or by change of reaction.

Adsorption in biochemic analysis, Abderhalden's Handb., 6, 100.

EXERCISE I.—(DEMONSTRATION) ADSORPTION BY INDIFFERENT SOLIDS

Fill a 6- to 10-inch percolator with dry sand, and tap to pack the sand. Pour on to this a 1 : 100,000 solution of methylene-blue: the solution becomes decolorized as it passes through the sand.

EXERCISE II.—(DEMONSTRATION) ADSORPTION OF ALKALOIDS, ETC., BY CHARCOAL

Experiment 1. Strychnin.—Mix 10 c.c. of a 1 : 1000 solution of strychnin sulphate with 1 gm. of powdered wood charcoal in a flask, and shake occasionally during one-half hour. Filter, and compare the filtrate with the original solution:

(a) The filtrate is tasteless.

(b) It gives no precipitate with mercuric-potassium iodid.

Freshly calcined animal charcoal acts similarly, and also removes coloring matter, etc.

Experiment 2. Dyes.—Shake 20 c.c. of a 1.5' : 1000 solution of methylene-blue with 0.1 gm. of bone-black: complete decolorization should occur within one minute. (Test for quality of charcoal.)

EXERCISE III.—(DEMONSTRATION) ADSORPTION OF ALKALOIDS BY COLLOIDAL CARBON (CAMEL)

Proceed as in Exercise II, using about 1 gm. of caramel (burnt sugar) in place of the charcoal and leaving for only ten minutes: the filtrate does not precipitate mercuric-potassium iodid (Sabbatani, 1914, Arch. di Farmacol., 16, 518).

Purified Caramel.—Berenger, 1912, Amer. Jour. Pharm., 160; Beal and Zoller, 1914, Jour. Amer. Pharm. Assoc., 3, 495.

EXERCISE IV.—(DEMONSTRATION) ADSORPTION OF ALKALOIDS BY HYDROUS ALUMINUM SILICATE (FULLER'S EARTH; LLOYD'S REAGENT; ALKRESTA)

To 10 c.c. of an acid 1 : 1000 solution of quinin sulphate add about 1 gm. of the earth. Shake occasionally during ten minutes. Filter a few drops and test with Meyer's reagent: negative. Render the mixture alkaline with ammonia; shake with chloroform; evaporate a few drops of the chloroform solution and test with Meyer's reagent: positive.

The earth adsorbs alkaloids from acid or neutral solutions more rapidly than does bone-black. The adsorbed alkaloid is liberated by alkalies (Gordin and Kaplan, 1914, Jour. Amer. Pharm. Assoc., 3, 627; Fantus, 1914, Ibid., 3, 657; Rehfeld, *ibid.*, 710; J. U. Lloyd, 1918, *ibid.*, 5, 490). The earth also precipitates barium chlorid, lead acetate, zinc sulphate, etc.

TECHNICAL REFERENCES

Estimation of Adsorbing Efficiency of Fuller's Earth.—The decolorization of malachite green is used for this purpose (Fantus, 1915, Jour. Amer. Med. Assoc., 64, 1838).

EXERCISE V.—COLLOIDS ON TASTE

Compare the taste of the following, dissolved in water, with the same strength solutions in 10 per cent. starch paste:

1. Citric acid, 1 per cent.
2. Quassia, $\frac{1}{10}$ per cent.
3. Quinin bisulphate, $\frac{1}{10}$ per cent.
4. Sugar, 5 per cent.
5. Salt, 3 per cent.

QUESTIONS ON CHAPTER XVII

1. Define and explain adsorption.
2. What class of substances act as adsorbents?
3. What effect would the combination of an alkaloid with an adsorbent have on (a) its taste, (b) its systemic action?
4. What substances can be improved in taste by adsorbents?

CHAPTER XVIII**(DEMONSTRATION) SELECTIVE SOLVENTS**

The distribution of a substance between two solvents is determined by chemic and solution affinity and by adsorption. The selective affinity of drugs for cells is controlled by the same principles.

S. M.—Solutions for Exercise V.

EXERCISE I.—DISTRIBUTION COEFFICIENT

1. **Chloroform.**—In a 50-c.c. graduated cylinder place 20 c.c. of water, 20 c.c. of olive oil, and 10 c.c. of chloroform. Shake occasionally during fifteen minutes; let stand, and read the volume of the two solutions.

2. **Alcohol.**—Perform a similar experiment, using alcohol instead of chloroform.

3. **Ether.**—Use either instead of chloroform.

The distribution-coefficient = volume of substance dissolved in oil \div volume dissolved in water.

EXERCISE II.—ABSTRACTION OF PHENOL BY SOLVENTS

25-c.c. portions of a 1 per cent. aqueous solution of phenol are shaken occasionally during fifteen minutes with the following solvents. A little of the aqueous solution is decanted, and the intensity of the color given by ferric chlorid is compared with that of the original phenol solution:

1. Kerosene oil.
2. Olive oil.
3. Turpentine.
4. Ether.

EXERCISE III.—VOLUME OF SOLVENTS

Shake 10-c.c. portions of a saturated aqueous solution of iodine with the following. Compare the depth of color of the iodine in both layers:

- (a) 10 c.c. chloroform.
- (b) 50 c.c. chloroform.
- (c) Five successive 10-c.c. portions of chloroform.

EXERCISE IV.—COMPETITION OF SOLVENTS

Add a little dry starch to 1 : 10,000 solutions of iodine in—

- (a) Water.
- (b) Alcohol.
- (c) Chloroform.

Shake and let settle until clear. Note changes in the color of the starch and of the solvent.

EXERCISE V.—INTERMEDIARY SOLVENT

Shake some powdered iodine with the following. Compare the depth of color:

- (a) Water.
- (b) 25 per cent. alcohol.

EXERCISE VI.—DISTRIBUTION BY CHEMICAL AFFINITY

Shake 10 c.c. of a dilute iodine solution in chloroform with the following. Note the color of the chloroform solution:

- (a) Water.
- (b) 5 per cent. NaOH.

EXERCISE VII.—(SPECIAL ASSIGNMENT) EVAPORATION OF ANESTHETIC MIXTURES

Take the specific gravity of a mixture of equal parts of chloroform and ether. Evaporate one portion (a) spontaneously and another (b) by a brisk current of air. Control the specific gravity when one-quarter, one-half, and three-quarters have been evaporated.

TECHNICAL REFERENCES

Vital Staining.—L. B. Wilson, 1915, Jour. Lab. Clin. Med., 1, 40.

QUESTIONS ON CHAPTER XVIII

1. PARTITION COEFFICIENT OF ANESTHETICS

- (a) What is the partition coefficient of chloroform, alcohol, and ether?
- (b) The membrane and contents of nerve-cells are rich in lipoids. On the assumption that the anesthetic action is conditioned on the lipoid content, what would be the order of efficiency of the chloroform, alcohol, and ether? Does this conform to the facts?

2. EXTRACTION OF PHENOL BY SOLVENTS

- (a) What is the order of solubility of phenol in the fluids?
- (b) What bearing has this on the treatment of phenol poisoning?
- (c) Would an oily solution of phenol be antiseptic? Why?

3. VOLUME OF SOLVENT

- (a) Is a given quantity of solvent more effective if used in one or in several fractions? Why?
- (b) Would it be possible to remove iodine from chloroform by means of water? Why?

4. COMPETITION OF SOLVENTS

Arrange the solutions in the order of the intensity of the blue color. Assuming that the blue color is due to solution of iodine in the starch, explain why the intensity of the starch reaction is unequal in the different solutions.

5. INTERMEDIARY SOLVENT

- (a) Why does the solution take up more iodine in the presence of alcohol?
- (b) Explain the possible bearing of this observation on the fact that the activity of a substance (in this case, the coloring power) may be increased by a second substance that does not itself possess this power (potentiated synergism; amboceptor group).

6. DISTRIBUTION BY CHEMICAL AFFINITY

- (a) Why is the iodine removed from chloroform by NaOH and not by water?
- (b) Explain the possible bearing of this observation on the fact that the pharmacologic activity of a substance may be diminished (or increased) by the addition of a second substance that may itself be inactive.

7. EVAPORATION OF ANESTHETIC MIXTURES

- (a) Does the specific gravity remain constant during the evaporation?
- (b) Does the composition remain constant?
- (c) In what way would this interfere with the administration of anesthetic mixtures?

CHAPTER XIX

(DEMONSTRATION) OSMOSIS AND DIFFUSION

Explanatory.—The protoplasm of cells takes up water and swells when they are placed in dilute solutions; while it loses water and shrinks when they are placed in strong solutions of salts, and indeed, of most soluble substances. This process is called *osmosis*. In order that osmosis may occur it is necessary that the two solutions (in this case the protoplasm and the salt solution) have a different concentration; and that they are separated by a membrane (the cell wall) which is permeable to water, but not to the dissolved molecules. A membrane of this kind is called *semipermeable*. A membrane which is not quite impermeable to the dissolved molecules but which interposes more resistance to them than it does to water, may be termed *partly semipermeable*. Most, if not all, cell walls belong to the last class; so does parchment. These membranes often possess a different degree of permeability for different salts.

The molecules of a substance in the state of solution behave precisely like the molecules in a gas (Van't Hoff's Theory), and obey the same laws (Gay-Lussac's, Avogadro's, Boyle-Mariotte's). They therefore tend to distribute themselves evenly through the space at their disposal, *i. e.*, through the solvent. When they are prevented from doing so by the interposition of a semi or partly semipermeable membrane, they exert a pressure which is strictly proportional to the number of molecules present in a unit of space, and independent of the nature of the molecules. This is called the *osmotic pressure*.

A *mol* (molecular weight expressed in grams) dissolved in a liter of water exerts the same pressure as a mol of gas confined in the same space, *i. e.*, 22.34 atmospheres at 0° C. This osmotic pressure can only be realized under the above conditions—*i. e.*, when two solutions are separated by a semipermeable membrane. If the two solutions have the same *molecular concentration* (mols per liter), they will be under the same osmotic pressure; they are said to be *isotonic*. If they are of different concentration, the stronger solution will be under a higher pressure; it is said to be *hyperisotonic*; the weaker is *hypo-isotonic*. This difference of pressure tends to equalize itself by the passage of the solvent through the membrane, so as to render the two solutions of equal concentration. This changes the volume of the solutions: the weaker solution diminishes, the stronger gains, in volume. This is the explanation of the changes in the volume of the cells.

The law that the osmotic pressure is directly proportional to the molecular concentration holds strictly only for substances like urea, alcohol, sugar, etc. It needs to be modified for acids, bases, and salts; for in dilute solutions the molecules of these substances fall apart, the fragments acquiring charges of electricity, and being known as *ions* (Arrhenius' Hypothesis). The degree of ionization increases with dilution. Each ion behaves physically like an entire molecule. A very dilute solution of NaCl therefore exerts twice

the calculated osmotic pressure; sulphuric acid ($\text{H}-\overset{+}{\text{H}}-\overset{+}{\text{S}}\text{O}_4$) three times; sodium phosphate ($\text{Na}-\overset{+}{\text{Na}}-\overset{+}{\text{H}}-\overset{-}{\text{P}}\text{O}_4$) four times, etc. (The + and — indicate the nature of the electric charge which is carried by the ion.) The undissociated molecules and the ions, existing in a solution under given conditions, are called collectively *mol-ions*. It is really the mol-ions, and not the mols, which determine the osmotic pressure.

The experimental determination of the absolute osmotic pressure is beset with serious technic difficulties. It requires the construction of a vessel with strictly semipermeable walls, of sufficient strength to withstand the high pressure.

The Pfeffer cell is the nearest approach; a porous clay cell is filled with copper sulphate and set in a solution of potassium ferrocyanid. The two solutions meet in the pores, and cause a precipitate of the reddish-brown copper ferrocyanid, which functionates as a semipermeable membrane. *Osmometers*, thistle-shaped tubes closed with parchment, bladder, or peritoneal membrane, are useful in certain physiologic experiments; but they are only partly semipermeable.

Fortunately, there are other properties of solutions which vary precisely with the molecular concentration, and which are much more easily determined. Such are the boiling-point or, most conveniently, the freezing-point. Each mol-ion, added to a liter of water, depresses the freezing-point of the water by exactly 1.85° C. (Raoult's Law). This depression of freezing-point is denoted by Δ . A 1 per cent. NaCl solution gives Δ 0.589.

Osmotic Pressure Through Partly Semipermeable Membranes.—It is evident that this cannot reach the theoretic level; for some of the molecules will escape. If the membrane is as permeable to the dissolved molecules as it is to water, there can be no osmotic pressure whatsoever, no matter what the concentration. Such a solution will therefore be hypo-isotonic to a solution the dissolved molecules of which cannot pass the mem-

brane. One may therefore see the paradoxical phenomenon of a weaker solution (of a non-permeating substance) being hyperisotonic to a stronger solution (of a permeating substance). The law that *equimolecular solutions* (having the same molecular concentration) are isotonic holds therefore only for strictly semipermeable membranes. The cell membrane of the red blood-corpuscles is strictly semipermeable to most substances. The corpuscles are therefore isotonic to a 0.9 per cent. NaCl solution, and to equimolecular solutions of most other substances. Urea and ammonium salts are exceptions; they penetrate readily, and their solutions are consequently hypo-isotonic and produce laking. Many other cells (for instance, those of the kidney) show more numerous peculiarities of penetration.

Solutions of substances with very large molecules always exert a low osmotic pressure, since even the strongest solutions must have a low molecular concentration. To this class belong the *colloids*—gums, proteins, gelatin, etc.

Osmosis is most conspicuous with the substances of small molecular weight, the crystalloids. It is most important in the case of salts; the subject of osmosis is therefore often called **SALT-ACTION**.

EXERCISE I.—DIFFUSION INTO AGAR

Pour a hot 2 per cent. solution of washed agar into two test-tubes until they are half filled. Let cool and set. Fill one test-tube with a solution of copper sulphate or methylene-blue (crystalloid substances); the other with a solution of Congo-red (a colloid). Let stand one or two days. The true solution (methylene-blue) will have diffused through the agar; the colloid solution (Congo) will present a fairly sharp line of separation. (Some samples of Congo diffuse freely.)

TECHNICAL REFERENCES

Diffusion Coefficient.—Tigerstedt, 1, 2, 202.

Dialysis.—Abderhalden's Handb., 3, 10, 165; Golodetz, 1913, Zs. physiol. Chem., 86, 315.

Collodion Membranes.—Hawk, Physiol. Chem., 30; Abel, 1914 (tubes), Jour. Pharmacol., 5, 275; Beal, 1914, Jour. Amer. Pharm. Assoc., 3, 499; Lillie, 1907, Soc. Exp. Biol. Med., 4, 111; Meigs, 1913 (with calcium phosphate), *ibid.*, 10, 129; Schoep, 1911, Zentr. Bioch. Bioph., 11, 377 (glycerin to increase porosity; castor oil for elasticity); Meigs, 1915 (also porous cups, phosphate, and ferrocyanid membranes), Amer. Jour. Physiol., 38, 456.

Capsules, Browne and Soletsky, 1914, Sci., 40, 176.

Ultrafiltration.—Abderhalden's Handb., 5, 1086; Zsigmondi, 1913, Zbl. Bioch. Bioph., 15, 849; Gaucher, 1912, Chem. Abst., 7, 618.

EXERCISE II.—INCREASE OF VOLUME AND PRESSURE BY OSMOSIS

1. **Osmometers**.—Fill the bulb of a thistle tube with syrup. Tie a wet parchment membrane over the bulb; immerse in a beaker of water, and note the height of the liquid in the tube from time to time. Lengthen the tube with another joint of tubing as necessary. The rise of the liquid shows increase of volume and pressure, the parchment acting as a partly semipermeable membrane.

2. (Optional) **Egg Experiment**.—Remove the shell from the broad pole of an egg without injuring the inner skin. Cement a short glass tube to the narrow end with wax; when a tight joint has been made, pierce the shell through the tube with a hat-pin. Join another piece of tubing, and stand the egg upright in a beaker of water. The fluid rises, the egg-skin acting as a partly semipermeable membrane.

TECHNICAL REFERENCES

Direct Determination of Osmotic Pressure.—Abderhalden's Handb., 1, 513; Tigerstedt, 1, 2, 136.

EXERCISE III.—(SPECIAL ASSIGNMENT)

Osmotic Changes in the Weight of Tissues.—Place the following solutions into evaporating dishes:

- a.* Water.
- b.* 5 per cent. NaCl.
- c.* 1 per cent. NaCl.
- d.* Urea, 1.89 per cent.
- e.* Sodium Citrate, 2.74 per cent. of anhydrous.

} Of the same freezing-point as 1 per cent. NaCl.

Cut a fresh dog's or rabbit's kidney into sections about 1 mm. thick. Rinse a section in 1 per cent. NaCl for a moment, dry it superficially with filter-paper, and weigh; lay it in solution *a*. Prepare other sections in the same manner, laying them in the other solutions. Leave in the solutions for half an hour, then again dry and weigh the sections. The weights will be changed, the sections having absorbed or lost water through osmosis:

- (*a*) Increase of weight—water being strongly hypo-isotonic.
- (*b*) Decrease of weight—5 per cent. NaCl being strongly hyperisotonic.
- (*c*) Increase of weight—The protoplasm of the kidney cells is therefore hyperisotonic to 1 per cent. NaCl (and consequently to blood-serum). It requires about 1.8 per cent. of NaCl to keep the weight unchanged.
- (*d*) Increase of weight—much larger than in (*c*). Consequently, the kidney cells are easily permeable to urea.
- (*e*) Decrease of weight—The sodium citrate penetrates less readily than sodium chlorid.

The experiment illustrates strikingly that the osmotic pressure depends not only on the molecular concentration, but also on the permeability of the cell wall, which is different for each substance in the kidney. Urea penetrates readily, chlorid less, and citrate still less so.

TECHNICAL REFERENCES

Abderhalden, 3, 542, 547; D. Cohnheim, 1913, *Zs. physiol. Chem.*, 84, 481; Ehrenberg, 1913, *Arch. ges. Physiol.*, 153, 1; Hirokawa, 1908, *Beitr. chem. Physiol.*, 11, 458.

EXERCISE IV.—PASSAGE OF FLUID BY SOLUTION-AFFINITY (L'HERMITE EXPERIMENT)

In a graduated 50-c.c. cylinder (stoppered) place, without mixing, 25 c.c. of chloroform, 3 c.c. of water, and 22 c.c. of ether. Let stand a week and longer, observing the layers.

EXERCISE V.—“OSMOTIC PRESSURE” BY SOLUTION-AFFINITY

Fill a very thin rubber balloon (condom) with olive oil. Tie a long glass tube in the opening, and immerse into a cylinder filled with ether. Observe from time to time. The liquid rises in the tube just as in an osmometer (W. J. Gies, etc., 1912; *Bioch. Bul.*, 2, 55).

EXERCISE VI.—ALTERATIONS IN MEMBRANE PERMEABILITY

Into 10-cm. segments of fresh dog's intestine (ligated at both ends) place 10 c.c. of 2 per cent. NaCl, containing the reagents named below. Place each loop in a large test-tube containing equal amount of water, sufficient to cover the loop.

Let stand two hours; remove the water, and test it for chlorid with silver nitrate and nitric acid. Compare the intensity of the Cl reaction in the dialysates.

- (a) 2 per cent. NaCl as control.
- The other tubes, each 2 per cent. NaCl, with the following additions:
- (b) Lactic Acid, 0.5 per cent.
- (c) Sodium Carbonate, 0.5 per cent.
- (d) Calcium Nitrate, 0.5 per cent.
- (e) Mercuric Chlorid, 0.1 per cent.
- (f) Picric Acid, saturated.
- (g) Salicylic Acid, 0.3 per cent.
- (h) Magnesium Sulphate, 5 per cent.
- (i) Phenol, 1 per cent.

QUESTIONS

1. Which agents increase the permeability?
2. Which agents diminish the permeability?
3. Suggest explanations.

EXERCISE VII.—IMBIBITION

Lay plates of dry gelatin, about 1 cm. square, into the following liquids, and observe after an hour or longer whether they have swollen:

- (a) Water.
- (b) Oil.
- (c) Absolute alcohol.
- (d) 50 per cent. alcohol.
- (e) 25 per cent. alcohol.

QUESTIONS

- (a) Arrange the plates in the order of swelling.
- (b) Explain the cause of the differences.

TECHNICAL REFERENCES

Tigerstedt, 1, 2, 4, 209; M. Fischer, *Edema*, 1910 (Wiley and Sons).

EXERCISE VIII.—CHEMIC CHANGES BY ADSORPTION

Pack a wide glass tube loosely with absorbent cotton. Immerse one end into a solution of Congo-red, rendered slightly acid. In a few minutes the cotton immediately above the solution will be colored blue (acid reaction); above this red (neutral or alkaline reaction); above this it will be wet but colorless. The cotton, therefore, adsorbs the acid ions first, then the alkali ions (E. G. Parker, 1913, *Jour. Agric. Res.*, 1, 179).

QUESTIONS

- (a) Are all the ions of a salt absorbed equally?
- (b) How may this affect chemic processes?

QUESTIONS ON CHAPTER XIX

- (a) What causes the molecules of the methylene-blue to move through the agar?
- (b) Why cannot the Congo move in the same way?
- (c) Do all kinds of crystalloids diffuse through all kinds of colloids?
- (d) What would happen if the passage of the methylene-blue were impeded, while water could pass freely?

- (e) What causes the water to rise in the osmometer or egg?
- (f) What would happen if the membrane were impermeable alike to the solvent and solute?
- (g) What changes would a semipermeable cell undergo when laid in (a) a hypotonic; (b) a hypertonic solution?
- (h) Would the change be strictly proportional to the molecular concentration when different solutes are compared? Why?
- (i) What is Van't Hoff's explanation of the nature of osmotic pressure?
- (j) What other explanation does the L'Hermite and Gies experiments suggest?
- (k) Why does the ether pass into the chloroform and into the oil; and not vice versa?
- (l) Is the permeability of a membrane constant, or may it alter?
- (m) Why do the gelatin plates swell in some liquids and not in others?
- (n) What are the differences between imbibition and osmosis?
- (o) How may adsorption affect chemic reactions?

CHAPTER XX

(OPTIONAL) DETERMINATION OF MOLECULAR CONCENTRATION

Explanatory.—Since osmotic effects depend on molecular concentration, the determination of this concentration is important. With pure solutions of non-electrolytes the concentration can be computed from the percentage of the dissolved substance (gm. per L. divided by molecular weight = molecular concentration). With electrolytes a correction must be applied to allow for dissociation.

The total molecular concentration can easily be determined experimentally either by the depression of freezing-point, or by comparing the effect of the solutions on cells surrounded by a semipermeable membrane; for instance, by determining the relative concentration required to produce laking of red blood-corpuscles.

EXERCISE I, A.—(OPTIONAL) HAMBURGER'S BLOOD-CORPUSCLE METHOD

Blood-corpuscles are laked when placed in a solution of a certain concentration (about 0.525 per cent. NaCl); the relative concentration of solutions may, therefore, be determined by comparing them with a known sodium chlorid solution. This holds true only if the blood-corpuscles are equally impermeable to the observed substance. It may be accepted as correct for most substances, with the notable exceptions of urea and ammonium salts.

Prepare solutions of NaCl, NaNO₃, and Urea, all having the same freezing-point (1 per cent. NaCl; 1.535 per cent. NaNO₃; 1.89 per cent. Urea). Set up a series of clean test-tubes, of about 15-c.c. capacity and of equal diameter. With a pipet, graduated accurately in $\frac{1}{10}$ c.c., place in the first 4 c.c. of the NaCl solution and 6 c.c. of water; in the second, 4.5 c.c. NaCl and 5.5 of water; third, 5 c.c. and 5 c.c.; fourth, 5.5 c.c. NaCl and 4.5 c.c. water; fifth, 6 c.c. NaCl and 4 c.c. water. Place corresponding dilutions of NaNO₃ and of urea in the other tubes. Mix the contents of each tube. Add to each 10 drops of defibrinated blood. Let stand overnight.¹ Note the tube in each series in which there is just perceptible laking. This will be the same for the chlorid and nitrate, but all the urea tubes will be laked.

¹ The preceding part of the experiment should be prepared on the previous day; only the results of the experiment being demonstrated.

EXERCISE I, B.—(OPTIONAL) PLASMOLYSIS

Experiments similar to those with corpuscles may be made with various plant cells. Haskins (p. 50) used red beet.

TECHNICAL REFERENCES

Blood-corpuscle and Plasmolytic Methods.—Abderhalden's Handb., 1, 513; 6, 83; Tigerstedt, 1.2, 179; Heinz, 1, 34, 37.

Osmotic Resistance of Corpuscles.—Stewart, 73. With normal dog's blood, hemolysis begins with 0.462 per cent. NaCl; and is complete with 0.33 per cent.

Hematocrit.—Stewart, 68; Heinz, 1, 39.

Permeability of Cells, determination.—Abderhalden, 3, 545.

EXERCISE II.—(OPTIONAL) DETERMINATION OF FREEZING-POINT

This is done by the Beckmann apparatus (Fig. 5). This consists of a thermometer (g), with an arbitrary scale (which must be adjusted for each determination, see below) graduated in 0.01°C . This is supported by a cork in a large strong test-tube (e), which may bear a side piece (f) for the introduction of ice. The cork is perforated for a platinum stirrer (h). The test-tube is supported in a larger tube (d), which acts as an air jacket, equalizing the temperature. This sits in a jar (a) of freezing mixture, together with a stirrer (c) and ordinary thermometer. The principle of the method consists in overcooling the contents of the test-tube until ice forms, when the thermometer column suddenly rises and comes to a standstill at the correct freezing-point. The zero point is first controlled by the standard sodium chlorid solution (10 gm. of dried salt dissolved in 1 liter of water, $\Delta = 0.589$).

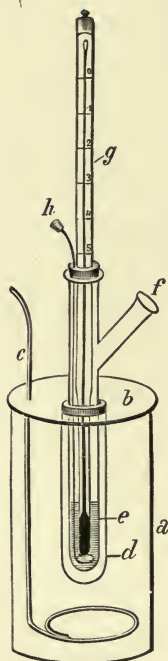


Fig. 5.—Beckmann-Heidenhain's apparatus for determining the freezing-point of a solution.

ings must be subtracted. The Δ of defibrinated blood and a sample of urine may now be determined.

TECHNICAL REFERENCES

Freezing-point Determination.—Abderhalden's Handb., 1, 485; 5, 328; 6, 355; 8, 419; Tigerstedt, 1.2, 140; Heinz, 1, 42; Stewart, 492; Haskins, 51; Burian and Drucker, 1910 (for 1.5 c.c.), Centr. Physiol., 23, 772; Bartley's *Freezing-point Apparatus*, Mathews, Physiol. Chem., p. 201.

Boiling-point Determination.—Abderhalden, 6, 364; 8, 434.

Melting-point Determination.—Ibid., 8, 419.

Micro-determination of Molecular Weight.—Barger, 1904, Trans. Chem. Soc., 86, 286; 1915, Abderhalden, 8, 1.

DEDUCTIONS FROM DEPRESSIONS OF FREEZING-POINT (Δ)

The freezing-point can be used for the following calculations:

1. The Molecular Concentration = $\frac{\Delta}{1.85}$
2. The Osmotic Pressure = $\frac{\Delta \times 1697.8}{1.85} \times (1 + \frac{t^\circ}{273})$ cm. of mercury or $\frac{\Delta \times 22.34}{1.85} \times (1 + \frac{t^\circ}{273})$ atmospheres (t° = temperature in degrees centigrade).
3. The Molecular Weight = $\frac{1.85 \times \text{gm. per liter}}{\Delta}$ (if no ionization occurs).
4. The Dissociation Coefficient (factor i) = $\frac{\Delta \times \text{molecular weight}}{1.85 \times \text{gm. per liter}}$. (This factor gives the ratio on mol-ions to mols. It is used for deducing the actual freezing-point or molecular concentration from that which is calculated on the assumption that no dissociation occurred.)
5. The proportion of ionized molecules (factor a) = $\frac{i-1}{n-1}$, i being the factor of the last paragraph; n , the largest number of ions into which the molecules can split (2 for NaCl, 3 for Na₂SO₄, etc.).

EXERCISE III.—(OPTIONAL) DETERMINATION OF IONIZATION BY ELECTRIC CONDUCTIVITY

With a Kohlrausch apparatus determine the conductivity of a NaCl solution. Note that this is relatively greater the more the solution is diluted, until a constant is finally reached.

TECHNICAL REFERENCES

Measurement of Conductivity (also in blood, etc.).—Abderhalden, 1, 484; Tigerstedt, 1, 2, 161; Heinz, 1, 46; Stewart, 68.

QUESTIONS ON CHAPTER XX

1. Name three methods of determining the molecular concentration of a solution of sugar. Would these give concordant results?
2. How would the results of these methods differ when applied to a solution of urea? Why?
3. How and why would they differ with NaCl?
4. Explain the Arrhenius hypothesis.
5. What is meant by dissociation coefficient?
6. Work out the following problems:
 - (a) What is the molecular concentration of blood-serum, if $\Delta = 0.555$?
 - (b) What is its osmotic pressure at 38° C.?
 - (c) What is the molecular weight of urea, if a 2 per cent. solution = $\Delta 0.62$?
 - (d) What is the dissociation coefficient of a 1 per cent. NaCl solution ($\Delta 0.589$; molecular weight, 58.4)?
 - (e) What fraction of the molecules is ionized?

CHAPTER XXI

AGGREGATION OF COLLOIDS

Explanatory.—The size of colloid particles is intimately concerned in their solution or precipitation, surface tension, adsorption, viscosity, and consistency, etc., and, therefore, in their physiologic properties. This size, in turn, depends mainly on the *electric charges* of the particles: increase of the electric charges tends to make the particles fly apart and thus become smaller; and vice versa. Electrolytes, therefore, produce important effects on the properties of colloids.

The affinity of the particles for the solvent is also important. **Suspension colloids** (for instance, the colloid metals, Prussian blue, etc.) do not take up the solvent and, therefore, remain discrete. The **emulsion colloids**, which comprise most of the proteins and are, therefore, especially important in biology, have a strong affinity for water, and adsorb it with considerable force ("**imbibition**"; for instance, when dry gelatin is laid in water). When the quantity of solvent is limited the emulsion colloids form "gels"; even solutions have a high viscosity.

TECHNICAL REFERENCES

- Properties of Colloids.*—Ostwald, Colloidchemie, Dresden, 1911; Abderhalden, 1, 508.
Ultramicroscope.—Abderhalden, 1, 283.
Refractometer.—Abderhalden, 1, 568; 8, 84; Tigerstedt, 1.2, 224.
Surface Tension (see also Viscosity).—Michaelis, Dynamik der Oberflaechen, Dresden, 1909; Tigerstedt, 1.2, 220; Morgan, 1911, Jour. Amer. Chem. Soc., 32, 349.
Viscosity.—Abderhalden, 5, 1358; Tigerstedt, 1.2, 212; Erdmann, 1913, Jour. Biol. Chem., 14, 141; *of blood*, Abderhalden, 3, 743; Burton-Opitz, 1911, Jour. Amer. Med. Assoc., 57, 353; Huertle, 1900, Arch. ges. Physiol., 82, 415.
Nephelometer.—Marriott, 1913, Jour. Biol. Chem., 16, 290; Richards, 1895, Zs. anorg. Chem., 8, 269; Richards and Wells, 1904, Amer. Chem. Jour., 31, 235; Bloor, 1915, Jour. Biol. Chem., 22, 145 (conversion of Duboscq into nephelometer).

EXERCISE I.—(DEMONSTRATION) VISCOSITY OF SUSPENSION AND EMULSION COLLOIDS

The viscosity can be judged by the time required for a given column to run through a certain orifice.

Time the outflow from the same 10-c.c. pipet of:

- (a) Water.
 - (b) Colloidal ferric hydroxid (Merck's dialyzed iron).
 - (c) 10 per cent. dilution of egg white.
 - (d) 10 per cent. dilution of egg white containing 1.5 per cent. of NaI.
 - (e) 10 per cent. dilution of egg white containing 0.6 per cent. of NaCl.
- (d and e are m/10 solutions; the percentages of m. solutions in this chapter refer to the grams of anhydrous substance added to 100 c.c. of water.)

EXERCISE II.—(DEMONSTRATION) GELATINIZATION

Cool a warm 5 per cent. solution of gelatin under the tap: it sets into a jelly. Heat: it becomes liquid.

Repeat the experiment with a 5 per cent. gelatin solution containing m/10 of the following:

- (a) Cane-sugar, 3.4 per cent.
- (b) NaI, 1.5 per cent.
- (c) Na₂SO₄, 1.4 per cent., anhydrous.
- (b) does not gelatinize on cooling.

EXERCISE III.—(OPTIONAL) ELECTROLYTES ON HEAT COAGULATION

Use a 10 per cent. dilution of egg white, which has been dialyzed in a parchment tube against distilled water for several days. To 5-c.c. portions add 5 c.c. of the following, and heat to boiling:

- (a) Water.
- (b) m solution of cane-sugar (34 per cent.).
- (c) m solution of NaCl (5.8 per cent.).

Only the last coagulates.

EXERCISE IV.—(OPTIONAL) IONS ON ELECTRONEGATIVE COLLOIDS

To 5-c.c. portions of the dialyzed egg solution (which is electronegative) add 2 c.c. of each of the following, and let stand several hours if necessary:

- (a) m/20 CaCl_2 , 0.55 per cent.
- (b) m/20 MgCl_2 , 0.47 per cent.
- (c) m/100 MnCl_2 , 0.13 per cent.
- (d) 2m KCl, 14.9 per cent.
- (e) 2m NaCl, 11.7 per cent.
- (f) 2m LiCl, 8.5 per cent.

(a), (b), and (c) are precipitated; (d), (e), and (f) not.

EXERCISE V.—(OPTIONAL) IONS ON ELECTROPOSITIVE COLLOIDS

To the dialyzed egg solution add m/50 HCl (0.07 per cent.) until completely precipitated; then again the same quantity of HCl. With this solution repeat Exercise IV, (a), (c), (d), and (e). The reactions are reversed.

Suspension colloids of opposite electric charges also precipitate each other.

EXERCISE VI.—(OPTIONAL) INTERFERENCE OF ELECTROLYTES

To 5-c.c. portions of the dialyzed egg solution add—

- (a) 5 c.c. of m KI (16.6 per cent.) + 2 c.c. m/20 MgCl_2 (0.47 per cent.): no precipitation.
- (b) 5 c.c. of water + 2 c.c. m/20 MgCl_2 (0.47 per cent.): precipitation.

QUESTIONS ON CHAPTER XXI

- (a) Describe the characteristic differences between emulsion and suspension colloids.
- (b) Why is the aggregation of colloids affected by salts and not by sugar?
- (c) Why are electronegative colloids precipitated by bivalent cations, and not by monovalent?
- (d) Why is this reversed by the addition of HCl?
- (e) What effects have NaI and Na_2SO_4 on the fluidity and viscosity of gelatin? Why do these salts differ in their effect?

CHAPTER XXII**HEMOLYSIS, CRENATION, AND AGGLUTINATION OF RED BLOOD-CORPUSCLES**

If the experiments of this chapter have been performed in other courses they need not be repeated, but they should be read and the questions answered.

Explanatory.—A. Hemolysis (laking) consists essentially in a solution of the corpuscles; after a preliminary swelling, the hemoglobin and salts pass from the corpuscles into the serum (which therefore becomes colored). The stroma can at first be distinguished, especially by staining, as colorless “ghosts,” floating in the amber-colored fluid. These also are eventually dissolved.

Laking agents act by increasing the permeability of the cell envelope. This consists largely of fatty (lipoid) substances, especially lecithin and cholesterin. All *fat solvents*—ether, alkalies, saponin, etc.—therefore produce laking. The bacterial *hemolysins* probably act analogous to saponin.

The entrance of *water* into the cell also causes laking. This occurs when the cell is laid in water or in any solution of a weaker salt-content than serum. The result is due to osmosis.

B. Stronger salt solutions, on the other hand, withdraw water from the cell and shrivel it, producing "**crenation**."

C. Agglutination consists in the clumping of corpuscles. It is probably due to a change in the viscosity of the envelope. It may be produced by dilute acid and some other chemic agents, but is seen most typically with certain toxins, the *agglutinins*.

EXERCISE I.—TEST-TUBE EXPERIMENTS ON HEMOLYSIS

(Students may work in groups of four.)

Put into 8 *perfectly clean* and *dry* test-tubes:

(a) 5 c.c. of 0.9 per cent. sodium chlorid.

(b) 5 c.c. of 0.9 per cent. sodium chlorid containing $\frac{1}{10}$ per cent. of crude saponin.

(c) 5 c.c. of 0.9 per cent. sodium chlorid containing $\frac{1}{10}$ per cent. of crude saponin which has been digested for an hour at 40° C. with 2 drops of 1 per cent. cholesterin solution in ether.

(d) 5 c.c. of 0.9 per cent. sodium chlorid, saturated with ether.

(e) 5 c.c. of 0.9 per cent. sodium chlorid containing 1 per cent. of urea.

(f) 5 c.c. 1. per cent. urea.

(g) 5 c.c. 2 per cent. Na_2CO_3 .

(h) 5 c.c. of distilled water.

(e and f must be freshly prepared.)

Add to each tube 2 drops of defibrinated blood and shake. Observe after half an hour in which tube laking has taken place, as denoted by the clearness of the mixture or the color of the supernatant fluid.

Isotonic solution of sodium chlorid (a) is indifferent, and does not cause laking. The addition of sapotoxin (b) dissolves the fatty envelope, and thus allows laking. If cholesterin (c) is added, the sapotoxin is bound and cannot act on the corpuscles, and there is no laking. (In the body the cholesterin of the blood acts as a protective in this way.) Ether (d) and other fat solvents, as also alkalies (g) also cause laking by dissolving the fatty envelope. Water (h) injures the corpuscles by removing the salts. Urea (f), to which the corpuscles are permeable, acts like water. In either case the addition of salt in isotonic proportion (a and e) prevents laking.

(Optional).—*Carmin-fibrin* is said to behave to hemolytic agents similarly to blood (M. H. Fischer, 1909, Koll. Zs., 5, 146).

TECHNICAL REFERENCES

Hemolysis Technic.—Abderhalden's Handb., 5, 24; Fuehner, Nachweiss, 32; Hemolysis and Agglutinin Experiments, Stewart, 70; *Antigen*, Abderhalden, 3, 1191; *Osmotic resistance of corpuscles*, Stewart, 73.

Bio-estimation of Saponin.—Kobert, 1910, Yearbk. Amer. Pharm. Assoc., 1, 446.

Antihemolytic Action of Tea Infusions Against Saponin.—This has been proposed as a test of genuine tea (Maggiara and Ferron; ref., Zentr. Bioch. Bioph., 18, 199).

Sodium Oleate Hemolysis; inhibition by cholesterin; cholesterin—oleate solutions: O. Klotz and Bothwell, 1915, Soc. exp. Biol. Med., 12, 199.

Blood-corpuscles.—Abderhalden, 5, 143; Kobert, Intox., 1, 158; *Stroma*, Abderhalden, 5, 146; *Ratio to plasma*, ibid., 3, 538; 5, 148; *Blood-count*, Tigerstedt, 2.5, 1; Heinz, 1, 374; Abderhalden, 3, 714. Data in different animals: J. J. Wells and Sutton, 1915, Amer. Jour. Physiol., 39, 31.

S. M.—Materials for Exercise I.

Platelets.—Abderhalden, 5, 144; 6, 383; Tigerstedt, 2, 5, 134.

Experiments on Blood.—Abderhalden, 5, 21.

Drawing of Blood.—Shaffer, 1914, Jour. Biol. Chem., 19, 297; Abderhalden, 3, 1186; 5, 23; 7, 721; Tigerstedt, 1, 2, 116.

Determination of Blood Quantity.—Schürer, 1911, Arch. Exp. Path. Pharm., 66, 171.

EXERCISE II.—MICROSCOPIC CHANGES IN BLOOD-CORPUSCLES

1. **Saponin-laking**.—Place a small drop of defibrinated blood, diluted with 10 volumes of 0.9 per cent. NaCl, on a slide under a cover-glass. Examine with the medium power of the microscope. Add to one edge a drop of 2 per cent. saponin in 0.9 per cent. NaCl, strongly tinged with methylene-blue. It will be seen that the corpuscles swell, then lose their hemoglobin; but the stroma ("ghosts") remains for a considerable time, and can be discerned faintly by the methylene-blue stain.

2. **Water-laking**.—Repeat the last experiment, but add water tinged with methylene-blue in place of the saponin solution: the corpuscles are seen to swell and to lose their hemoglobin, but more slowly than with the saponin.

3. **Amyl Alcohol**.—Repeat the experiment, adding amyl alcohol in place of the water. Move the cover-glass a little: the corpuscles become agglutinated into small clumps and then lose their hemoglobin.

4. **Crenation**.—To a drop of defibrinated blood under the microscope add a drop of saturated salt solution: the corpuscles shrivel and become crenated by the abstraction of water. Similar phenomena can be seen in most cells.

5. **Agglutination by Ricin**.—On one end of a slide place a rather large drop of 0.9 per cent. NaCl; on the other end, a similar drop of 0.1 per cent. ricin in 0.9 per cent. NaCl. Add to each a small drop of defibrinated blood, cover, and examine with the microscope after fifteen minutes: The corpuscles in the ricin solution will be "agglutinated" into clumps. Many toxins, and the sera of foreign species, have a similar action.

TECHNICAL REFERENCES

Agglutination.—Abderhalden's Handb., 5, 28; Jour. Lab. Clin. Med., 1, 56, 1915; Stewart, 7, 70; *Ricin*, W. W. Ford, 1913, Centr. Bact., 58, 139.

QUESTIONS ON CHAPTER XXII

- (a) Which substances are hemolytic, and which are not?
- (b) What would be the results of injecting water rapidly into a vein?
- (c) Would the slow intravenous injection of a saturated solution of ether in 0.9 per cent. NaCl result in hemolysis? Why?
- (d) Would a 1 per cent. solution of glucose produce hemolysis? (The molecular weight of glucose is 180.)
- (e) Would a large dose of urea, taken by mouth, produce laking?

CHAPTER XXIII

(OPTIONAL) ANTIBODIES

The following experiments illustrate the main principles; but they need not be repeated if they have been studied in other courses.

EXERCISE I.—FOREIGN SERUM

1. **Hemolysis by Foreign Serum**.—(a) Wash rabbit's corpuscles with 0.9 per cent. NaCl, and add sufficient 0.9 per cent. NaCl to make a 5 per cent. suspension.

To 1 c.c. of this suspension add 0.5 per cent. of dog or ox serum; to another portion add 0.5 per cent. of 0.9 per cent. NaCl. Incubate at 40° C. for about two hours, when the corpuscles will be found laked by the serum.

(b) Make a similar experiment, using dog or ox corpuscles and rabbit serum: no laking.

2. **Destruction of Complement.**—Heat some dog or ox serum at 56°C . for one-half hour. Repeat experiment 1 (*a*), using this serum in place of fresh serum: no laking. Something essential to hemolysis has been destroyed by the heating (*viz.*, complement). Save this material for Experiments 3 and 5.

3. **Presence of Complement in Rabbit Serum.**—To one-half of the complement-free mixture from Experiment 2 add 0.2 c.c. of rabbit serum; incubate: laking occurs.

4. **Removal of Amboceptor.**—Centrifugalize 5 c.c. of the 5 per cent. washed rabbit corpuscles suspension. Pour away the supernatant saline and cool the corpuscles to 0°C . Add 0.5 c.c. of dog or ox serum, also cooled to 0°C ., and keep at this temperature for one hour. Centrifugalize rapidly, and separate the serum and corpuscles (keep the corpuscles for Experiment 6).

To 0.02 c.c. of the 5 per cent. suspension of the original washed rabbit corpuscles add 0.1 c.c. of this serum and incubate. Little or no laking occurs because the first corpuscles have removed the amboceptor from the serum.

5. **Mixture of Amboceptor and Complement.**—Add 0.1 c.c. of the serum of Experiment 4 (which contains complement but no amboceptor) to some of the mixture of Experiment 2 (which contains amboceptor but no complement): laking occurs on incubation.

6. **Demonstration of Fixed Amboceptor in Rabbit Corpuscles.**—Wash the corpuscles from Experiment 4 with cooled 0.9 NaCl. To the separated corpuscles add some of the serum of Experiment 4 (which contains complement but no amboceptor): laking occurs, showing that the corpuscles had fixed the amboceptor.

EXERCISE II.—PRODUCTION OF PRECIPITINS AND HEMOLYSINS BY IMMUNIZATION

Inject intraperitoneally into a rabbit 5 to 10 c.c. of defibrinated ox (or dog) blood. Repeat the injection twice, at intervals of six to seven days each. A week or longer after the last injection obtain some serum from the rabbit. This contains hemolysin for the blood-corpuscles and precipitin for the serum of the ox (or dog). These are not present in the serum of normal rabbits.

1. **Hemolysin.**—Repeat Exercise I, 1, using ox and dog corpuscles with the serum of the treated and of a normal rabbit: laking occurs only with the corpuscles for which the rabbit has been immunized.

2. **Precipitin.**—Make a series of dilutions of dog and ox serum with 1 to 1000 parts of 0.9 per cent. NaCl. To 0.5 c.c. of these dilutions add 0.2 c.c. of the immunized rabbit serum, and keep at 40°C .: turbidity, followed by precipitation, occurs in the serum toward which the rabbit has been immunized.

TECHNICAL REFERENCES

Immunology.—Abderhalden's Handb., 3, 1185; Tigerstedt, 2, 1, 48; Zinnser, Hopkins, and Ottenberg, Laboratory Course in Serum Study; *Precipitins*, Abderhalden, 3, 1185; 7, 538; Jour. Lab. Clin. Med., 1915, 1, 56.

QUESTIONS ON CHAPTER XXIII

- (a) How can it be shown that two distinct substances are necessary for serum-hemolysis?
- (b) Why does not the dog serum lake the dog corpuscles?
- (c) Why does not the rabbit serum lake the dog corpuscles?
- (d) How can a serum be deprived of its complement?
- (e) How can one restore the activity of such a serum?
- (f) How can the amboceptor be removed from a serum?
- (g) What becomes of it?
- (h) How can it be shown that it has not been destroyed?
- (i) Is the rabbit incapable of manufacturing amboceptors? Why?
- (j) How could you show whether the hemolysin and precipitin in Exercise II are identical?

CHAPTER XXIV

(OPTIONAL) EFFECTS OF DRUGS ON HEMOGLOBIN

These experiments need not be repeated if they have been performed in other courses.

Explanatory.—The blood pigment, hemoglobin, gives a characteristic absorption spectrum (Fig. 6). It is easily altered by chemic reagents, with corresponding modifications in the spectrum. This is sometimes important in diagnosing poisoning.

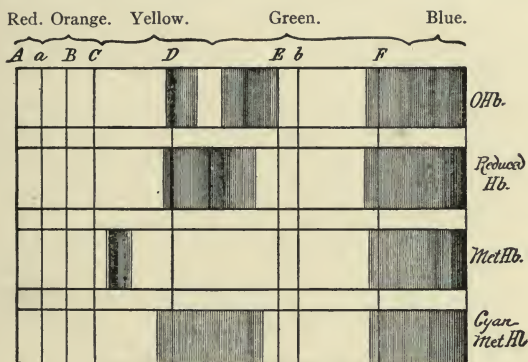


Fig. 6.—Spectroscopic bands of blood pigments.

TECHNICAL REFERENCES

Experiments on Hemoglobin.—Stewart, 74; Tigerstedt, 21, 68; Kobert, *Intox.*, 1, 94, 163, 273; *Spectra*, Abderhalden's Handb., 6, 389; Heinz, 1, 389; *Estimation*, Abderhalden, 3, 749; Heinz, 1, 377; Haldane, 1901, *Jour. Physiol.*, 26, 497; Haessler and Newcomer, 1916, *Arch. Int. Med.*, 17, 806; Kuttner, 1916, *Jour. Amer. Med. Assoc.*, 66, 1370; *Crystals*, Abderhalden, 5, 203; distinction for species, Reichert and Brown, 1908, *Soc. Exp. Biol. Med.*, 5, 66; *Spectroscopy*, Abderhalden, 1, 609.

Influence of Sex and Age.—Williamson, 1915, *Jour. Amer. Med. Assoc.*, 65, 302.

Chemic Tests for Blood.—Lenhartz, 183, 271; Kastle, 1909, *Hyg. Lab. Bul.* 51; Merck's Rep., 25, 434; 27, 484; A. L. Holland, 1913, *Med. Rec.*, Oct.; *Benzidin reaction*, McWeeney, 1909, *Zbl. Bioch. Bioph.*, 10, 320; Bordas, *ibid.*, Lyle, Curtman, and Marshall, 1914, *Jour. Biol. Chem.*, 19, 445; *Phenolphthalein test*, Meyer, 1908, *ref.*, *Amer. Pharm. Assoc.*, 57, 398; Merck's Rep., 23, 285; *Guaiaec Test*, Holland, 1907, *Jour. Amer. Med. Assoc.*, 48, 1942; *In feces*, Abderhalden's Handb., 5, 394; Dewis, 1907, *Bost. Med. Surg. Jour.*, 157, 169.

EXERCISE I.—OXYHEMOGLOBIN AND REDUCED HEMOGLOBIN

Use a solution of 4 parts of defibrinated blood¹ in 100 parts of water or of $\frac{1}{10}$ per cent. NaOH.

1. **Oxyhemoglobin.**—Place some of the solution in a test-tube and examine with the spectroscope and note the two dark lines, between yellow and green (diluting if necessary).

2. **Reduced Hemoglobin.**—Add a few drops of fresh ammonium sulphid to the test-tube: notice the darker color and observe the single band.

EXERCISE II.—CARBON MONOXID HEMOGLOBIN

1. **Spectroscopic Test.**—Pass some coal-gas through a tube of the diluted blood. The spectrum is almost unchanged. The color is a deeper, brighter red. Add a few drops of the sulphid: the double band persists; there is no reduction.

Carbon Monoxid, which is the principal toxic ingredient of coal-gas, acts by combining so firmly with hemoglobin that it cannot take up oxygen. Death, therefore,

¹ Dog's blood contains on an average 15 per cent. of hemoglobin; beef's blood, 10 per cent.

occurs by asphyxiation. The combination is broken up by a great excess of oxygen, so that recovery is possible with artificial respiration or the inhalation of oxygen.

The skin and mucous membranes are of a bright, cherry red color in carbonic oxid poisoning; whereas they are blue in ordinary asphyxia.

2. **Color Test.**—The color of the blood itself is the most certain proof of carbonic oxid poisoning. The test is performed as follows:

Add a drop of undiluted blood to each of two test-tubes half filled with water. Pass a stream of coal-gas through one of the tubes, and note that the color changes from amber to carmin. In suspected poisoning, a drop of blood is drawn from the finger, diluted as in the above, and compared with the control tube. The depth of the red color permits an approximate estimate of the degree to which the hemoglobin is saturated with CO.

3. **Chemic Tests.**—These depend for the most part on the addition of substances that change the color of oxyhemoglobin but not of CO-hemoglobin.

(a) **NaOH:** To a 1 : 20 dilution of blood add an equal volume of 30 per cent. NaOH: CO blood remains light red; normal blood changes to dirty brown.

(b) **Hydrogen Sulphid.**—To a 1 : 50 dilution of blood add an equal volume of saturated H₂S water: CO blood shows no change; normal blood turns dirty green.

(c) **Ferrocyanid.**—Mix 10 c.c. of blood with 15 c.c. of 20 per cent. potassium ferrocyanid and 2 c.c. of 30 per cent. acetic acid: CO blood remains red; normal blood changes.

(d) **Tannin.**—Shake a 1 : 5 dilution of blood with 3 volumes of 1 per cent. tannin: CO blood is carmin red; normal blood turns gray.

4. **Demonstration of CO in Air.**—Shake 2 to 5 c.c. of diluted blood (just sufficient to give spectrum) in a liter flask containing the suspected air; or aspirate 10 liters of the air through diluted blood. Examine the blood for CO-hemoglobin, as in the preceding experiments.

TECHNICAL REFERENCES

Proof in Blood.—Gadamer, 43; Sand, 1914, ref., Jour. Amer. Med. Assoc., 63, 1890; *Estimation*, Abderhalden, 3, 637; Brunck, 1912, Chem. Abstr., 7, 747.

Preparation of CO.—Abderhalden, 3, 735; *Work with Gases*, Abderhalden, 1, 215, 230; 5, 1027; 8, 437.

EXERCISE III.—METHEMOGLOBIN

1. **Formation of Methemoglobin.**—Put some of the diluted blood (about 15 c.c.) into a series of six test-tubes. Add the reagents mentioned below, and note changes in color and spectrum at once. If none appear, place in a water-bath at 40° C. and observe every half-hour:

- (1) 25 drops saturated KClO₃.
- (2) 25 drops 5 per cent. Pot. ferricyanid.
- (3) 25 drops 10 per cent. NaNO₂.
- (4) 25 drops 1 per cent. KMnO₄.
- (5) 25 drops Phenylhydrazin.
- (6) 25 drops 10 per cent. Pyrogallol (Methemoglobin spectrum and precipitate of Hemogallol).

Methemoglobin has a rather brown color and shows a sharp band in the red, closely resembling acid hematin (see Fig. 6).

To one of the test-tubes which shows a good methemoglobin band, add a little ammonium sulphid: the reduction occurs comparatively slowly, and more of the reagent is required.

Explanatory.—Methemoglobin is a peculiar modification of oxyhemoglobin. It differs from the latter in being less readily reduced. The conversion of any considerable proportion of the blood pigment into methemoglobin therefore leads to asphyxia, characterized by intense cyanosis. This conversion takes place even more readily in the body than in the test-tube; the chlorate and the coal-tar products are especially apt to produce the effect in living mammals, while they act sluggishly on shed blood.

The conversion of hemoglobin into methemoglobin can be effected by: Oxidizing agents (1, 2, 4), reducing agents (3, 6), coal-tar derivatives (5). The rapidity of the conversion varies considerably: in 2, 3, and 4 it is almost instantaneous; in 1 it may require several hours; the others are intermediate. The results are somewhat different in the intact mammals, ClO₃ and the coal-tar products being quite active; (5) may also show the band of reduced hemoglobin.

2. **Cyan-hemoglobin.**—Add a drop of 2 per cent. hydrocyanic acid to some of the diluted blood and to some methemoglobin solution. The first shows no change. In the second, the color brightens and the spectrum changes so as to resemble reduced hemoglobin (see Fig. 6). This reaction may be used as a test for hydrocyanic acid or for methemoglobin.

This peculiar combination of cyan and hemoglobin does not occur normally during life, since the blood does not contain methemoglobin. The latter may be formed after death, especially in ecchymotic areas; and the bright red color of these spots is a characteristic feature of cyanid poisoning.

EXERCISE IV.—HEMATIN

The blood in the vessels does not show acid or alkali hematin even in severe poisoning, but they may be discovered locally; *e. g.*, in the vomit.

1. **Alkali Hematin.**—Add a few drops of sodium hydrate to the diluted blood. The color deepens; the spectrum changes to a broad, diffuse band.

2. **Acid Hematin.**—Add a little dilute acid to the diluted blood: the color becomes brownish, and some precipitation may occur. The spectrum shows a sharp line in the red.

3. **Hemochromogen.**—Add yellow ammonium sulphid to the hematin solution: the spectrum shows two bands in the green, the left much stronger.

TECHNICAL REFERENCES

Hematin, Abderhalden's Handb., 2, 617.

EXERCISE V.—HEMATOPORPHYRIN

Add a few drops of blood to sufficient concentrated sulphuric acid to be transparent: the spectrum shows two bands in the orange and yellow.

Hematoporphyrin does not contain iron. It occurs in the urine after sulphonal poisoning and can be extracted with amyl alcohol.

TECHNICAL REFERENCES

Determination in Urine.—Abderhalden's Handb., 3, 861.

QUESTIONS ON CHAPTER XXIV

- (a) Make a diagram of the spectra of all the compounds studied.
- (b) How would you distinguish between oxyhemoglobin and reduced hemoglobin?
- (c) Describe three characteristic tests for CO in blood.
- (d) How would you distinguish between acid hematin and methemoglobin?

CHAPTER XXV

CHEMIC EFFECTS OF CORROSIVES AND IRRITANTS

Explanatory.—All substances which enter directly into chemic combinations with proteins produce local effects, *i. e.*, they act at the place where they are applied. The action results in inflammation; these substances are therefore *irritants*; if the action is at all violent the cells are killed. If the combination of the reagent and protoplasm is fluid the tissue is dissolved. This is termed *corrosion* or *cauterization*. If, on the other hand, the action is mild and the product insoluble, the effect is *astringent*, *i. e.*, mucous membranes are constricted and puckered, and the phenomena of a pre-existing inflammation are lessened. These precipitates also serve to stop the lumen of bleeding vessels and are, therefore, *styptic* or *hemostatic*.

It is therefore important to know whether the action of these agents results in precipitation or solution. This may be studied on isolated proteins. It must be remembered, however, that the effects depend greatly upon the concentration of the reagent: the precipitates often redissolve in an excess of the reagent or of the protein.

The *color* of the compounds is often important in diagnosis.

The application of the corrosives to *excised tissues* shows that these influence the effect; the skin is generally more resistant than the softer structures. The tissues also illustrate the stains and the penetration of the corrosion.

(Students may work in groups of four.)

TECHNICAL REFERENCES

Investigation of Irritants, Heinz, 1, 255.

EXERCISE I.—PROTEINS (EGG-ALBUMEN)

Place in each of twelve test-tubes $\frac{1}{2}$ inch of a solution of egg-albumen (the white of 2 eggs to 200 c.c. of water, strained). Add the following reagents (the usual solutions), drop by drop:

(1) HgCl_2 ; (2) AgNO_3 ; (3) CuSO_4 ; (4) Fe_2Cl_6 (tincture); (5) Lead Acetate; (6) H_2SO_4 ; (7) HCl ; (8) HNO_3 ; (9) NaOH ; (10) Carbolic Acid (strong); (11) Alcohol; (12) Tannin (6, 7, 8, 10, and 11: full strength).

A white precipitate is given by HgCl_2 , AgNO_3 , $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$, H_2SO_4 , HCl , $\text{C}_6\text{H}_6\text{O}$, $\text{C}_2\text{H}_6\text{O}$, and Tannin; a greenish-white precipitate by CuSO_4 ; a yellowish-brown precipitate by Fe_2Cl_6 ; a yellow precipitate by HNO_3 . NaOH gives no precipitate.

Excess of the reagent redissolves the precipitate in the case of acids, but not with the other precipitants. (The reagents which redissolve the precipitate are apt to penetrate more deeply into tissues.)

QUESTIONS

(a) Tabulate the results as to: Precipitate, its firmness or absence; color changes; resolution of the precipitate in the reagent.

(b) Which of the reagents would tend to penetrate deepest?

(c) Which would tend to be superficial?

(d) Which would tend to be corrosive; and which astringent?

(e) Which cause characteristic color changes?

EXERCISE II.—DEFIBRINATED BLOOD

Place about 2 c.c. of defibrinated blood in twelve test-tubes, and add the reagents as in Exercise I.

A black or brown clot is formed with Fe_2Cl_6 , H_2SO_4 , and HNO_3 ; a brown or dark precipitate with CuSO_4 , HCl , and NaOH ; a pink or light red precipitate with $\text{C}_6\text{H}_6\text{O}$, $\text{C}_2\text{H}_6\text{O}$, and Tannin; a gray precipitate with HgCl_2 , PbAc_2 , and AgNO_3 . Excess of the reagent redissolves the precipitate with a brownish-red color in the case of acids, and with a garnet color in the case of soda. The others do not redissolve. (The color is due to acid hematin in the case of acid, to alkali hematin in the case of NaOH .)

QUESTIONS

(a) Tabulate the results as to: Precipitation and its firmness; color changes; solution in excess of the reagent.

(b) Which of these agents would tend to be hemostatic?

(c) Which would give "coffee-grounds" vomit?

S. M.—Egg-albumen.

EXERCISE III.—CORROSION OF SKIN

Place bits of fresh mammalian skin into test-tubes containing concentrated H_2SO_4 , HCl , HNO_3 , NaOH , and $\text{C}_6\text{H}_6\text{O}$. Leave for fifteen minutes, rinse in water, and note the effect on the hair and on the epithelial and connective-tissue surfaces.

With the acids, the *epithelial surface* becomes first white, hard, and somewhat shrunken. With more prolonged action it is gradually softened. With HCl it remains white; HNO_3 , light yellow; H_2SO_4 , brownish. $\text{C}_6\text{H}_6\text{O}$ causes a more pronounced shrinking, puckering, and hardening, without subsequent softening. NaOH softens. The *hair* is softened and dissolved by the NaOH , more slowly by the acids. It is not affected by $\text{C}_6\text{H}_6\text{O}$. The *connective tissue* is rendered softer and transparent and is finally dissolved by the NaOH and the acids, and stained as the epithelium. The carbolic acid affects it as it does the epithelium.

QUESTIONS

- (a) Tabulate the results for epithelium, connective-tissue surface, and hair as to: softening or hardening; shrivelling; color changes.
- (b) Which of the agents could corrode the skin?
- (c) Which would cause extensive destruction?
- (d) Which would act as depilatories?
- (e) Which give characteristic stains?

EXERCISE IV.—CORROSION OF MUCOUS MEMBRANES

Slit open a piece of fresh dog's intestine, 3 inches long, and flatten it, epithelial surface up. With a glass rod apply a drop of the reagents used in Exercise III. Observe during fifteen minutes. Note the character, color, and depth of the effect. See whether the epithelium detaches more readily. The acids first turn the epithelium white and hard, but soon softer and darker. The underlying tissues appear white and hard, as if cooked. The epithelium is readily detached. The action extends deepest with HNO_3 . This gives a yellow tinge to the stain. H_2SO_4 gives a brownish color. $\text{C}_6\text{H}_6\text{O}$ acts as it does on the skin; its effect extends deeply. NaOH first softens the tissues and then renders them gelatinous. The epithelium scrapes off very readily.

QUESTIONS

- (a) Tabulate the results, both for the epithelium and underlying tissue, as to: hardening or softening; detachment of epithelium; depth of corrosion; color changes.
- (b) Arrange the reagents in the order of their liability to produce perforation.
- (c) Which would show characteristic stains at autopsy?

EXERCISE V.—CORROSION OF MUSCLE

Place bits of muscle into the reagents used in Exercise III, and observe during fifteen minutes. Rinse in water and note appearance and consistency.

H_2SO_4 and HCl soften the muscle without swelling it; the color becomes a deeper red; the muscle then gradually disintegrates, dissolving entirely,

S. M.—Defibrinated blood; skin.

with a garnet color, in the case of the H_2SO_4 . In HNO_3 the muscle shrinks and hardens, the color changing to yellow or brown, with partial solution. In $\text{C}_6\text{H}_6\text{O}$ the muscle is bleached, shrinks, and becomes hard, assuming a "cooked" appearance. NaOH causes the muscle to become red and swollen; the outer layers soften, become gelatinous, and dissolve to a red solution.

QUESTIONS

- (a) Tabulate the results as to: hardening or softening; swelling; solution; color changes.
- (b) What is the cause of the "cooked" appearance?

EXERCISE VI.—(OPTIONAL) COAGULATION OF MUSCLE

Tease a bit of fresh frog's muscle in normal saline on a slide, examine with the lower power of the microscope; add a drop of concentrated H_2SO_4 and observe the results. Repeat with the other reagents mentioned in Exercise III, and also with 1 per cent. caffeine.

The acids cause the fibers to shrivel and to become contorted; they turn granular and opaque, the striations are lost, and gradual solution occurs. $\text{C}_6\text{H}_6\text{O}$ acts at first like the acids, but there is no solution. NaOH causes the fibers to swell, to become transparent, and to gradually dissolve. Caffeine produces a granular opacity.

QUESTION

Tabulate the changes in the structure of the muscle-fibers.

EXERCISE VII.—STAINS ON HUMAN SKIN

The observations made on excised tissues apply also to the human skin. The stains may be removed in the manner indicated in the experiment.

1. Apply to the intact skin of the forearm a drop of *concentrated nitric acid*. Wash off as soon as there is itching. An intensely yellow stain develops. Apply a drop of ammonia: the stain turns to an orange brown (xanthoproteic reaction). It is very lasting and wears off only as the skin is desquamated.
2. Apply to another place a drop of saturated *picric acid*: yellow stain. Apply ammonia: the stain is removed.
3. Apply concentrated *sulphuric acid* and *hydrochloric acid* to different places; wash off as in 1: there is no stain, but redness.
4. Apply strong alcoholic solution of *methylene-blue*; wash off after one hour. The stain is not removed by water, but by rubbing with dilute ammonia.
5. Apply a drop of tincture of *iodin* to two places, leave for five minutes: mahogany stain which cannot be washed off. Apply ammonia to one of the spots and sodium thiosulphate solution to the other: the stains disappear.

QUESTIONS

- (a) Tabulate the results as to color of the stains; time of appearance; persistence.
- (b) Which reagents give a yellow stain? How can they be distinguished?
- (c) How can an iodine stain be removed? How can a silver stain be removed?

S. M.—Dog's intestine; muscle.

CHAPTER XXVI

PHYSIOLOGIC EFFECTS OF IRRITANTS

Explanatory.—The tissues respond to irritants by the phenomena of inflammation. Four successive stages may be recognized *in the skin*: (1) *Rubefaction*, or reddening with pain and itching; (2) *Vesication*, or blistering; (3) *Pustulation*, the formation of isolated pustules; and (4) *Corrosion*, or destruction of tissue. The degree of the action depends on the nature and the concentration of the irritant. The rapidity of action is also variable. Chloroform and turpentine, for instance, act quickly, but scarcely progress beyond rubefaction; cantharides, croton oil, and antimony act slowly, but progress, the first to vesication, the last two to pustulation. A quick action is generally associated with volatility. Vesication demands that the irritant should remain in the skin sufficiently long to produce an inflammatory exudate under the impermeable stratum corneum. Pustulations have a specific chemotactic power on leukocytes.

Mucous membranes show only rubefaction and corrosion, the anatomic conditions being unsuitable for vesication or pustulation. The mouth, however, is an exception, for vesication may occur here. Irritation of mucous membranes is also characterized by catarrh—*i. e.*, increased excretion of mucus. This is diminished by *astringents*. These also cause puckering.

The *treatment of irritation* consists in the removal of the irritant and the application of fats, glycerin, or mucilage. This may be studied on carbolic acid.

TECHNICAL REFERENCES

Determination and Comparison of Local Toxicity of Chemic Compounds.—Cooper, 1915, Proc. Amer. Soc. Biol. Chem., 3, 19.

EXERCISE I.—IRRITATION OF SKIN

1. **Rubefaction.**—(a) Rub a little *chloroform* on the arm. Note the burning and reddening.

(b) (Optional).—Apply a *mustard* paper to the chest until tingling occurs. Note the sensation and the reddening.

2. (Optional) **Vesication.**—Apply some cerate of *cantharides* (or 0.1 mg. of cantharidin in a drop of oil) to the arm. Cover with adhesive plaster and leave for six hours: a blister forms. Wash off the excess of the cantharis with alcohol.

3. (Demonstration) **Urticaria.**—A few drops of *Histamin*, 1 : 1000, are rubbed vigorously into the skin or, better, applied to a non-bleeding scratch; or 0.5 to 1 c.c. may be injected hypodermically. This produces a typical urticaria (Eppinger, 1913, Wien. med. Woch., No. 23). Similar wheals are raised by *morphin* (1 : 100) or its esters. *Epinephrin* (1 : 1000) produces intense blanching and "goose-flesh." *Veratrin* (1 : 10,000), produces intense shooting pain.

4. (Optional) **Pustulation.**—Apply a drop of a 25 per cent. solution of *croton oil* in cottonseed oil to the skin of the arm; a pustular eruption is developed after some time.

QUESTIONS

(a) Tabulate the effects as to intensity, onset, and duration.

(b) Which of the drugs are classed as rubefacients, vesicants, and pustulants?

(c) Can chloroform or mustard produce vesication?

EXERCISE II.—IRRITANTS ON MUCOUS MEMBRANES

1. Shake a bottle containing *soap-bark* and smell it; sneezing.
2. Place a drop of ten times diluted tincture of *aconite* on the tip of the tongue: persistent tingling sensation.
3. Place a drop of Tr. *Iodin* on inner surface of lip: blister.
4. Observe the *astringent taste* of a 5 per cent. solution of *alum*, of Tr. Ferri Chlor., and of Tannin.
5. (Optional).—Snuff a very little mixture of 1 part of *veratrin* and 500 parts of starch; sneezing and all the phenomena of acute coryza.
6. (Optional) **Quantitative Estimation of Aconite by Squibb's Taste Method.**—Details, Ford, Ford and Wine, 1915, Amer. Jour. Pharm., 87, 489.

QUESTIONS

- (a) Tabulate the observations.
- (b) Name some sternutatories (drugs producing sneezing).
- (c) Would iodine produce a blister in the stomach? Why?
- (d) Does the aconite produce inflammation? What is the difference?
- (e) Name some astringents.
- (f) For what conditions could they be useful?

EXERCISE III.—(DEMONSTRATION) TREATMENT OF IRRITATION (PHENOL)

1. **Effects of Solvents.**—Arrange five small beakers in a circle so that the fingers can be plunged into them simultaneously. Fill these beakers with 5 per cent. carbolic acid in (a) water; (b) 25 per cent. alcohol; (c) 25 per cent. glycerin; (d) turpentine; (e) cottonseed oil.

Insert the five fingers of the left hand, one in each solution; keep in for five minutes, withdraw, and note the blanching and wrinkling, the tingling (felt especially on pressing the fingers against a table), and the anesthesia.

The effects (especially the blanching) are greatest in the water; much less in the alcohol and glycerin; practically absent in the oil.

Rinse the finger which has been in the watery solution in a liberal quantity of water: the blanching persists. Rinse it in 95 per cent. alcohol: the blanching disappears.

2. (Optional) **Phenol Burns.**—Dip the tips of two fingers into undiluted liquefied carbolic acid for one minute. Very little burning is felt, but the skin becomes white. Now rinse the one finger in water, the other in 25 per cent. alcohol. The latter removes the blanching, but not the sensory phenomena. It is effective against the superficial actions, but not against those which are situated more deeply. Glycerin, oil, or turpentine act like alcohol. Rinse the other finger in the alcohol. There will be some subsequent roughening and chapping of the skin.

3. (Demonstration) **Solvents on Precipitation.**—Pour $\frac{1}{2}$ inch of undiluted egg-white into two test-tubes; pour over this (without mixing) in (a) an equal volume of 5 per cent. phenol in water; to (b) in oil; (a) precipitates at once, (b) very slowly. The phenol, being very soluble in oil, does not pass into the watery egg-white.

Explanatory.—The reagents (b) to (e) of Experiment 1 are all better solvents for carbolic acid than is the skin; they consequently lessen the penetration of the phenol and hence its effects (Experiment 3). (These solutions of phenol are therefore also much less efficient as antiseptics than are watery solutions.) The glycerin and cottonseed oil act in addition in

S. M.—Soap-bark; Tr. Aconite, diluted 1 : 10.

S. M.—Egg-white; 5 per cent. phenol in oil.

virtue of their viscosity (*i. e.*, as *emollients*), hindering the access of new layers of the solution to the skin. This makes them more effective in the treatment of carbolic burns; but, on the other hand, it hinders the washing off of the phenol. Lavage of the stomach with 10 per cent. alcohol is the best local treatment in internal carbolic acid poisoning. For burns on the skin the surface should be rinsed with the dilute alcohol and then dressed with glycerin or oil. This treatment does not lessen the effects of the carbolic acid which has been already absorbed (except that still present in the superficial layers).

QUESTIONS

- (a) Tabulate the results.
- (b) What would be the proper treatment of phenol burns?
- (c) How could these facts be utilized in the treatment of internal phenol poisoning?

CHAPTER XXVII

(OPTIONAL) CATHARTICS ON MAN

Personal experience with the effects of the common cathartics is very useful to the physician. Students are, therefore, advised to try the following drugs at weekly intervals or as occasion arises, and to report their results as to time of effect; color, consistence, size and number of stools; griping, etc. A set of the cathartics will be furnished on application.

LAXATIVES

Aloin, 0.15 gm. at bedtime.
Calomel, 0.15 gm. at bedtime.
Cascara, Arom. Fldext., 5 c.c. at bedtime.
Castor Oil, 5 c.c. at bedtime.
Epsom Salt, 5 gm. in glass of water before breakfast.
Petrolatum Liquid, 1 oz. at bedtime.
Phenolphthalein, 0.1 gm. at bedtime.
Podophyllum Resin, 0.01 gm. at bedtime.
Rhubarb, 1 gm. at bedtime.
Senna, 5 gm. at bedtime.

CATHARTICS

Castor Oil, tablespoon before breakfast.
Comp. Jalap Powder, 2 gm. before breakfast.
Epsom Salt, 15 gm. in half a glass of water before breakfast.
Jalap, 1 gm. before breakfast.

CHAPTER XXVIII

ANTISEPTICS

The relative efficiency of the different types of antiseptics under actual working conditions is fairly well illustrated by the following experiments.

TECHNICAL REFERENCES

- Standardization of Antiseptics*.—Anderson and McClintic, 1912, Hyg. Bul. No. 82; Abderhalden's Handb., 5, 9; Heinz, 1, 128.
Potassium Tellurite.—Use as indicator of bacterial life, etc., W. E. King and Davis, 1914, Amer. Jour. Publ. H., 4, 917.
Bacterial Cultures and Media.—Abderhalden, 3, 1212; 5, 584.

EXERCISE I.—(SPECIAL ASSIGNMENT) URINARY ANTISEPTICS

Empty the bladder before breakfast, and save the urine. Take one of the following drugs (which are assigned to different numbers of the class). Collect the urine at the end of one, two to three, and six to eight hours after the administration.

Divide the samples into three parts. One is left at its natural reaction; the second is rendered slightly acid with HCl; the third slightly alkaline with sodium carbonate.

Incubate the different samples (including the control urine) and observe after twelve to twenty-four hours for bacterial turbidity and ammoniacal odor. If these are absent, continue the incubation, examining daily.

1. Hexamethylenamin. 0.5 gm.
2. Sod. Salicylate. 1.0 gm.
3. Sod. Benzoate. 1.0 gm.
4. Creosote. 0.3 gm.
5. Methylene-blue. 0.2 gm.
6. Boric Acid. 0.5 gm.
7. Santal Oil. 0.5 gm.

QUESTIONS

Tabulate the results, arranging the drugs in the order of their efficiency, and grouping them according to whether they are fully effective, moderately effective, or ineffective.

EXERCISE II.—(SPECIAL ASSIGNMENT) INTESTINAL ANTISEPTICS

Mince a mixture of equal parts of fresh pancreas and duodenum and mix with a double volume of water. Place equal quantities (about 15 c.c.) in a series of test-tubes. Add to each 0.15 gm. of the respective drugs. Stopper the tubes and incubate at about 40° C. Observe daily, noting the presence and intensity of putrefactive odor.

1. Control.
2. Bismuth Subcarbonate.
3. Calcium Carbonate.
4. Calomel.
5. Charcoal.
6. Creosote.
7. Glutol (Formaldehyd gelatin).
8. Guaiacol Carbonate.
9. Salol.
10. Sod. Phenolsulphonate.
11. Sod. Salicylate.
12. Tannin.
13. Thymol.

QUESTIONS

Tabulate the results, arranging the drugs in the order of efficiency, and grouping them according to those which prevent putrefaction, those which retard it, and those which are ineffective.

EXERCISE III.—(DEMONSTRATION) CALOMEL ON BILE

Place in an incubator some bile in which a knife-point of calomel has been added, and another sample without this addition, for control. The color changes first in the latter sample.

EXERCISE IV.—(SPECIAL ASSIGNMENT) WOUND ANTISEPTICS (DUSTING-POWDERS)

Place 15 c.c. of fresh defibrinated blood in a series of test-tubes. Add to each 0.15 gm. of the respective drugs. Stopper the tubes and incubate at about 40° C. Observe daily, noting the odor. (Laking and the changes of color are also interesting.)

1. Control.
2. Acetanilid.
3. Betanaphthol.
4. Bismuth Betanaphtholate (Orphol).
5. Bismuth Subnitrate.
6. Boric Acid.
7. Calcium Carbonate.
8. Charcoal.
9. Glutol.
10. Iodoform.
11. Tannin.
12. Thymol Diiodid (Aristol).
13. Zinc Oxid.

QUESTIONS

Tabulate the results, arranging the drugs in the order of efficiency, and grouping them according to those which prevent putrefaction completely; almost completely; those which merely delay, and those which are inactive.

EXERCISE V.—(SPECIAL ASSIGNMENT) DRYING POWDERS

Mix 1 c.c. of defibrinated blood with 1 gm. of the powders in small dishes and note consistence.

1. Control.
2. Bismuth Subnitrate.
3. Boric Acid.
4. Calcium Carbonate.
5. Charcoal.
6. Kaolin.
7. Starch.
8. Talc.
9. Tannin.
10. Zinc Oxid.

QUESTION

Tabulate in the order of their efficiency as absorbents (for wound secretions, etc.).

EXERCISE VI.—(SPECIAL ASSIGNMENT) PENETRATION OF ANTISEPTICS

Place 5 c.c. of each of the following antiseptics in 10-cm. ligated loops of fresh intestine of rabbit or cat. Be sure that there is no leak. Rinse the segments in water and place in test-tubes, each with 10 c.c. of water.

At the end of twenty-four hours pour off the water and test for the antiseptics.

In testing, compare with the original solution diluted 100 times, and, if necessary, 50, 25, and 10 times.

Note what fraction of the antiseptic has passed through the intestine, assuming that the antiseptic would have been diluted four times if it had diffused equally through the fluid.

Antiseptic solutions.	Tests.	Reference	
		Chapter.	Exercise.
Phenol, 5 per cent.....	Ferric Chlorid.	VII	I
Compound Cresol Solution, 2 per cent.....	Ferric Chlorid.	VII	I
Salicylic Acid, saturated.....	Ferric Chlorid.	VII	IV
Mercuric Chlorid, 1 : 1000.....	Ammon. Sulphid.	IX	XI
KI to solution of precipitate.			
Silver Nitrate, 1 : 1000.....	Ammon. Sulphid.	IX	XIII
Tr. Iodin.....	Starch Paste.	XII	XIV
Formaldehyd, 1 : 5000.....	Jorissen.	VIII	XII
Alcohol, 70 per cent.....	Chromate.	VIII	I

TECHNICAL REFERENCES

Penetration of Antiseptics.—Cheyne's Method, ref., Keilty and Packer, 1915, Jour. Amer. Med. Assoc., 64, 2123; Kendall and Edwards, 1911, Jour. Infect. Dis., 8, 250.

QUESTIONS

Tabulate the results in the order of penetration, grouping them according to those which penetrate readily, with difficulty, and not at all.

CHAPTER XXIX**EFFECTS OF DRUGS ON FERMENTS**

Ferments are greatly influenced by conditions, and thus by chemic substances. However, the effects of drugs are not easily studied under natural conditions, and unless these are reproduced in detail the results have little value. They are not of great practical importance, since the drugs, under natural conditions, do not remain in sufficiently lengthy contact with the ferments to exert much effect. A few of the reactions, however, are of special interest.

Students may work in groups of four.

TECHNICAL REFERENCES

Experiments with Ferments.—Kobert, Intox., 1, 149; Tigerstedt, 2.2, 54; *Preparation*, Abderhalden, 3, 1; *Recognition and Estimation*, ibid., 3, 16; quantitative, 24; viscosity, Feldsteiner and Weyl, 1910, Soc. Exp. Biol. Med., 7, 61; *isolation from bacteria*, etc., Abderhalden, 3, 1254; *Abderhalden test*, Abderhalden, 6, 223.

Trikresol as *antiseptic*, Graves and Kober, 1914, Jour. Amer. Chem. Soc., 36, 751.

EXERCISE I.—COAGULATION OF MILK

In a series of tubes place 5 c.c. of milk, 5 drops of rennin, and 5 c.c. of the following reagents; incubate for fifteen to thirty minutes, and note the occurrence and character of the coagulum:

1. Water.
2. Barley decoction (10 per cent. pearl barley).
3. Pancreatin, 0.1 per cent.
4. Formaldehyd, 0.1 per cent.
5. Sodium Citrate, 1 per cent.

QUESTIONS

- (a) Describe the results.
- (b) State what practical use could be made of them.

TECHNICAL REFERENCES

Milk Analysis.—Abderhalden, 5, 421; 7, 170.
Preparation of Rennin.—Ibid., 3, 10; *Estimation*, Hammarsten, 1914, Zs. physiol. Chem., 92, 119; *Casein Estimation*, Arny and Pratt, 1906, Amer. Jour. Pharm., 78, 121; *Pasteurized Milk*, microscopic stain, Frost, 1915, Jour. Amer. Med. Assoc., 64, 821.

EXERCISE II.—(OPTIONAL) COAGULATION OF BLOOD

Run 10 c.c. of blood from the artery of a living animal into test-tubes containing 2.5 c.c. of the following reagents. Incubate at 40° C., and observe the rapidity and the firmness of the coagulation:

1. 0.9 per cent. NaCl (control).
2. Ammonium Oxalate, 1 per cent. in 0.9 per cent. NaCl.
3. Sod. Citrate, 5 per cent. in 0.9 per cent. NaCl.
4. Sod. Fluorid, 1.2 per cent.
5. Magnesium Sulphate, saturated.
6. HCN, 0.5 per cent. in 0.9 per cent. NaCl.
7. Formaldehyd, 1 per cent. in 0.9 per cent. NaCl.
8. Leech-head Extract in 0.9 per cent. NaCl.
9. Brain Extract (Kephalin) solution.

QUESTIONS

- (a) Record the results.
- (b) State the mechanism by which each of the agents hinders coagulation.

TECHNICAL REFERENCES

Experiments on Blood Coagulation.—Stewart, 62; Heinz, 1, 386; Abderhalden, 5, 223; Kobert, Intox., 1, 158; *Coagulation Time*, Cannon and Mendenhall, 1914, Amer. Jour. Physiol., 34, 225; Buerker, 1912, Arch. ges. Physiol., 149, 318.
Thrombin.—Howell, 1913, Amer. Jour. Physiol., 32, 264; Abderhalden, 5, 273.
Antithrombin.—Howell, 1914, Arch. Int. Med., 13, 76; test in blood, Hess, 1915, Jour. Exp. Med., 21, No. 4; Minot and Denny, 1916, Arch. Int. Med., 17, 101.
Fibrinogen.—Whipple, 1914, Amer. Jour. Physiol., 33, 50; Abderhalden, 5, 253, 271.
Examination of Blood.—Abderhalden, 3, 742; 5, 155; *Total Analysis*, ibid., 5, 209.
Dry Residue.—Ibid., 5, 155; Ash, ibid., 159; *Specific Gravity*, ibid., 3, 742; Lenhartz, 126.
Blood-serum.—Abderhalden, 5, 142; Separation from clot, Sakaguchi, 1912, Zentr. Bioch. Bioph., 13, 757.
Serum Proteins.—Refractometer determination, T. B. Robertson, Jour. Biol. Chem., 22, 233; 33, 325; Tranter and Rowe, 1915, Jour. Amer. Med. Assoc., 65, 1432; E. Reiss, 1913, Erg. inn. Med. und Kindh., 10, 531; 1915, D. Arch. Klin. Med., 117, 175.
Blood Plasma.—Obtaining, Abderhalden, 5, 139, 257, 262, 268.
Calculation of Total Blood in Body.—Abderhalden, 3, 748; Tigerstedt, 24, 308; Dreyer and Ray, 1910, Roy. Soc., 82 B, 545; Schürer, 1911, Arch. Exp. Path. Pharm., 66, 171.

Average Count, etc., for Dogs, Musser and Krumbhaar, 1914, *Fol. Hemat.*, 18; for various animals, J. J. Wells and Sutton, 1915, *Amer. Jour. Physiol.*, 39, 31.

Collection of Body Fluids.—Tigerstedt, 1, 2, 113.

Bleeding of Rabbits.—Cut edge of back of ear with razor. Put point of split writing pen into vein in cardiac direction (Zinsser).

EXERCISE III.—(DEMONSTRATION) TRANSFORMATION OF SULPHUR INTO SULPHIDS

Sulphur owes its irritant action on the skin and intestines to its gradual transformation into sulphids. This is effected, at least in part, by the proteins. It is not affected by heat, so that ferments are probably not involved. Throw some pieces of fresh intestine into 20 c.c. of boiling water. Strain into a small flask. Neutralize. Add a pinch of washed sulphur. Stopper, suspending a piece of lead acetate paper from the stopper. In another similar flask place some water, sulphur, and lead paper. In a third flask place some intestine and boiling water, with lead acetate paper, for control. Observe that after a time the paper in the intestine and sulphur flask becomes blackened through the evolution of sulphuretted hydrogen. (Other proteins give the same result. The experiment is not always successful.)

EXERCISE IV.—(DEMONSTRATION) OXIDASE

Guaiac resin assumes a blue color when oxidized. This oxidation occurs even when the resin is suspended in plain water, but very slowly. It is greatly accelerated by oxidizing ferments (oxidases), which are present in all living protoplasm. One may use diluted defibrinated blood, or potato peelings, or fresh lettuce leaves pounded with sand and water and strained. These are placed in test-tubes, with a drop of fresh guaiac tincture (U. S. P.). The poison solutions are then added and the depth of the blue color noted from time to time. Prussic acid is especially effective in retarding this oxidation. Caffeine hastens it somewhat. It is very greatly accelerated by hydrogen dioxide.

Place into a series of test-tubes equal quantities of potato pulp (peelings rubbed with water and strained). Add an equal quantity of the reagents and 20 drops of fresh Tr. Guaiac. Let stand and note the development of the blue color:

1. Water (control).
2. HCN, 1 per cent.
3. Quinin Hydrochlorid, 2 per cent.

QUESTIONS

- (a) Report the results, arranging them in the order of interference.
- (b) In the light of these results, what would be the probable actions of these agents on metabolism?

TECHNICAL REFERENCES

Preparation of Oxidases and Catalases.—Abderhalden's *Handb.*, 3, 42; *Measurement in Plant-juices*, Bunzel, 1914, *Jour. Biol. Chem.*, 17, 409; *Measurement of oxidation velocity*, Abderhalden, 8, 21; of CO_2 production velocity, *ibid.*, 8, 38.

Respiration of Excised Tissues.—Abderhalden's *Handb.*, 3, 444, 451, 460; Batelli and Stern, 1908, *Arch. intern. Pharmacod.*, 18, 217.

Guaiac as Reagent.—Schaer, 1913, *Pharm. Ztg.*, 63, 328, obtained the best results with resin extracted from guaiac wood by chloroform; next came the natural resin; and finally the alcoholic extract of guaiac wood.

EXERCISE V.—DIGESTIVE AND SIMILAR FERMENTS

TECHNICAL REFERENCES

- Diastase**, *Preparation*, Abderhalden, 3, 387; *quantitative*, *ibid.*, 6, 231; *in feces*, *ibid.*, 5, 404; *in feces and urine*, T. R. Brown, 1914, *Trans. Assoc. Amer. Phys.*, 29, 547.
- Saliva**, *Examination*, Abderhalden, 3, 257.
- Invertin**, *Preparation*, *ibid.*, 3, 7, 389.
- Pepsin**, *Preparation and Estimation*, Abderhalden, 3, 8; Givens, 1915 (Modified Rose method), U. S. Hyg. Lab. Bul. 101, 71.
- Papain**, *Standardization*, Heyl, 1914, *Amer. Jour. Pharm.*, 86, 542.
- Trypsin**, *Preparation and Estimation*, Abderhalden, 3, 9; Long and Barton, 1914, *Jour. Amer. Chem. Soc.*, 36, 2151; *in feces*, Abderhalden, 5, 397; *in gastric juice*, W. H. Spencer, 1915, *Jour. Biol. Chem.*, 21, 165; *Pancreatic Juice*, *ibid.*, 6, 488.
- Erepsin**, *in feces*, *ibid.*, 5, 404.
- Secretin**, *Preparation and Tests*, Abderhalden, 3, 205, 418; 6, 487; 7, 65; Launoy and Oechsli, 1913, *Zentr. Bioch. Bioph.*, 15, 82.
- Lipase**, *Preparation, from liver*, Abderhalden, 3, 403.
- Castor beans*, Taylor on Fermentation, 258; Falk and Sugiura, 1915, *Jour. Amer. Chem. Soc.*, 37, 217; *blood*, Whipple, J. H. H. Bul. 24, 357; *Quantitative*, Abderhalden, 3, 220, 223; *in blood, etc.*, *ibid.*, 8, 301.
- Urease**, Van Slyke and Cullen, 1914, *Jour. Amer. Med. Assoc.*, 62, 1558.
- Nuclease**, Nephelometry, Kober and Graves, 1914.
- Tissue Juice**, Wiechowski's Method, Abderhalden, 3, 282; *Beitr. Chem. Physiol.*, 9, 232, 247.
- Buchner Press*, Abderhalden, 3, 2.
- Autolysis**, Abderhalden's Handb., 3, 433; 5, 1259; *antiseptics on*, Court, 1915, *ref.*, *Zentr. Bioch. Bioph.*, 18, 190; *alcohol on*, Wells and Caldwell, 1914, *Jour. Biol. Chem.*, 19, 57.
- Metabolism of surviving organs**, *ibid.*, 3, 358; 5, 1215; *perfusion*, Tigerstedt, 14, 51.
- Isolation of proteolytic ferments, from Liver and organs**, Abderhalden, 3, 407; *from plants*, *ibid.*, 413.
- Digestion Products, Collection and Analysis**, Abderhalden, 6, 458.
- Proteolytic Digestion Products, Isolation and Determination**, Abderhalden, 3, 227.
- Proteoses**, Abderhalden, 2, 533; 6, 506; *isolation*, *ibid.*, 3, 239; *silk peptone*, *ibid.*, 5, 578.
- Polypeptids**, *ibid.*, 2, 529, 545.
- Cleavage Products**, *ibid.*, 2, 470.
- Amino-acids**, *ibid.*, 5, 1011, 6, 276; *Determination in urine*, *ibid.*, 3, 810; 5, 309; *quantitative*, *ibid.*, 2, 470, 510, 559; 3, 1346; *in blood*, *ibid.*, 5, 190; *gasometric*, *ibid.*, 5, 995.
- Leucin**, *Determination*, *ibid.*, 3, 810; 5, 357.
- Tyrosin**, *Determination*, *ibid.*, 3, 810; 5, 357; Folin and Denis, 1912, *Jour. Biol. Chem.*, 12, 245.
- Tryptophan**, *Isolation*, Abderhalden, 3, 246; *Cancer Test*, Weinstein, 1910, *Jour. Amer. Med. Assoc.*, 55, 1085.
- Gastric Contents**, Lenhartz, 254.
- Protein Hydrolysis**, Comparison of methods, Harding and MacLean, 1916, *Proc. Amer. Soc. Biol. Chem.*, 3, 15.

CHAPTER XXX

MONOCELLULAR ORGANISMS AND LEUKOCYTES

Explanatory.—Poisons are divided into two groups: (1) Those which kill all forms of living tissue to which they may be applied; and (2) those which act selectively, *i. e.*, which have a much stronger action on some tissues than on others. The first are called *general protoplasmic poisons*; the second, *muscle-nerve poisons*.¹

¹ Their action is not necessarily restricted to muscular and nervous tissue, as the name would imply. It may also be exerted on gland-cells, etc. The distinctive feature of the classification is that the action is selective.

The general protoplasmic poisons are again subdivided into those which act also on dead proteins—the corrosives—and those which act exclusively on living cells—protoplasmic poisons in the restricted sense.

The effects of general protoplasmic poisons are studied most conveniently on monocellular organisms.

The boundary between the general protoplasmic poisons and the muscle-nerve poisons is not sharply defined. Many of the typically selective poisons, such as strychnin, are toxic to all tissues when they are used in sufficient concentration. The protoplasmic poisons also show some specialization. Quinin, for instance, kills ameboid cells much more readily than it does bacteria. All protoplasmic poisons, however, are to some extent bactericidal; and all antiseptics can be counted in this group. The estimation of the antiseptic power of protoplasmic poisons belongs to the domain of bacteriology.

EXERCISE I.—(DEMONSTRATION) YEAST FERMENTATION

Rub a cake of compressed yeast with 100 c.c. of glucose solution. Measure portions of 10 c.c. into a series of test-tubes and add 10 c.c. of the following reagents. Transfer them to fermentation tubes (such as are used in the fermentation test for sugar). Incubate at about 30° C. for one to two hours and compare the amount of gas evolved.

1. Water (control).
2. Quinin Hydrochlorid, 0.5 per cent.
3. Strychnin Sulphate, 0.5 per cent.
4. Sod. Fluorid., 0.1 per cent.
5. HCN, 0.1 per cent.
6. Sod. Salicylate, 0.05 per cent.

QUESTION

Report the results, arranging them in the order of interference.

TECHNICAL REFERENCES

Experiments with Yeast, Fuehner, 16; *Kobert, Intox.*, 1, 152; *Measurement of Yeast Fermentation*, Abderhalden, 8, 42; *Preparation of Zymase*, Abderhalden, 3, 393.

EXERCISE II.—(OPTIONAL) PROTOZOA

Macerate a little hay in water for several days until infusoria are developed. Place a drop of the infusion on a slide and note with the microscope the movements of the infusoria. Place a drop on each of four slides; add to slide (a) a drop of $\frac{1}{2}$ per cent. quinin; (b) $\frac{1}{2}$ per cent. cocain; (c) $\frac{1}{2}$ per cent. strychnin; (d) $\frac{1}{10}$ per cent. HgCl₂; (e) caffein, 1 : 700; (f) NaOH, 1 : 4000. Cover with cover-glasses (interposing a hair to prevent pressure) and examine at once, and then every ten minutes. The HgCl₂ kills the infusoria at once, fixing them in their original elongated shape. The others act much more slowly; the movements become more sluggish, and finally the infusoria contract to round balls and die. The caffein and alkali cause characteristic structural changes.

The quinin kills first, then the cocain, and last the strychnin. The observations need not be continued after the animals in the cocain have died.

QUESTION

Record the results, arranging them in the order of toxicity.

TECHNICAL NOTES

Protoplasmic Poisons.—Heinz, 1, 192.

Protozoa.—Abderhalden, 5, 18; Tigerstedt, 1.2, 1; Kobert, Intox., 1, 150.

Amebic Dysentery, Propagation.—Sellards and Baetjer, 1914, ref., Jour. Amer. Med. Assoc., 63, 1789.

Trypanosomes.—Abderhalden, 5, 1371.

Syphilis, Rabbits.—Jacobi, 99.

Tissue Cultures.—Abderhalden, 5, 836; 6, 519; Smyth, 1914, Jour. Amer. Med. Assoc., 62, 1377; R. A. Lambert, 1916, Proc. Soc. Exp. Biol. Med., 13, 100; Rous and Jones, 1916, Suspensions of living cells, *ibid.*, 13, 73.

Transplantation of Organs.—Abderhalden, 5, 828; Tigerstedt, 2.4, 336.

EXERCISE III.—(OPTIONAL) QUININ ON EMIGRATION OF LEUKOCYTES

Dispose a frog for the observation of the mesenteric circulation. Apply some 1 per cent. solution of quinin hydrochloric to a limited space. Observe the effect. Place an unpoisoned portion in the field and inject 1 or 2 c.c. of the quinin solution in the dorsal lymph-sac. Continue the observation for one-half hour, if necessary. (See illustration in Text-book.)

TECHNICAL NOTES

Experiments on Leukocytes.—Kobert, Intox., 1, 156; Tigerstedt, 2.5, 104; *Obtaining from blood*, Abderhalden, 5, 144; Zinsser, Hopkins, and Ottenberg, "Serum Study," 166; *Glycogenin*, *ibid.*, 5, 207; *Leukocyte count*, dogs, Musser and Krumbhaar, 1914, Fol. Hematol., 18.

Emigration, Ikeda, 1916, Jour. Pharmacol., 8, 101.

Phagocytosis, in vivo, F. C. Mann, 1916, Jour. Amer. Med. Assoc., 67, 174; *in vitro*, H. J. Hamburger, Brit. Med. Jour., Jan., 1916.

Opsonic Index.—Zinsser, Hopkins, and Ottenberg, "Serum Study," 168.

Chemotaxis.—*Ibid.*, 5, 1286; Ruchlaedew, 1910, Zs. Biol., 54, 533.

Light, actions, Abderhalden, 7, 587; determination of intensity, *ibid.*, 6, 180.

Fluorescence, Methods.—*Ibid.*, 3, 1171; experiments on animals, *ibid.*, 5, 563; in toxicologic analysis, Gadamer, 358.

Radio-activity.—Abderhalden, 7, 788.

CHAPTER XXXI

ANTHELMINTICS AND INSECTICIDES

The efficiency of the worm-remedies can be studied outside of the body.

EXERCISE I.—(DEMONSTRATION) ASCARIS

These occur in the pig and may be obtained from the slaughter-house. They keep alive for several days in a solution containing 1 per cent. NaCl and 0.1 per cent. sodium carbonate (Bunge's solution) if the temperature is kept constant at 37° to 38° C. At this temperature they are in constant movement. The action of the principal vermifuge, santonin, is not to kill the worm, but to increase its movements in the endeavor to escape from the santonin solution (v. Schroeder, 1895, Arch. exp. Path. Pharm., 19, 290).

1. Place the worms in beakers containing the reagents dissolved in the Bunge solution, at 37° C.

(a) Mercuric Chlorid, 0.1 per cent.

(b) Santonin, to saturation.

(c) Chenopodium Oil, 1 : 5000.

2. Fill a spiral glass tube with Bunge solution at 37° C. Add the worms and keep at this temperature. When they have assumed a fairly constant

position in the tube, add a concentrated solution of sodium santoninate to the open end of the tube: the worms generally move away as the santonin diffuses into the solution (W. Straub).

QUESTION

Describe the results.

EXERCISE II.—(DEMONSTRATION) ASPIDIUM

This is used for tapeworms, but its activity is tested most conveniently on the common rain worms.

1. Triturate 1 gm. of Oleoresin of Malefern with 2 gm. of calcined magnesia until dry. Mix with 10 c.c. of water, let stand a day, decant, and filter.

With a syringe and fine needle inject 0.1 c.c. of the solution into a large rain worm, just back of the clitellum. Keep the worm in a little water in a Petri dish, and observe from time to time: the segments around the injection swell and flatten. In three to four hours they liquefy.

TECHNICAL REFERENCES

Estimation of Efficiency of Anthelmintics.—Fuehner, 43; Bruening, 1906, Zs. exp. Path., 3, 564; S. Yagi, 1914, Zs. Exp. Med., 3, 64; *Effect of Anthelmintics on rain worms, leeches, and Ascaris*, Trendelenburg, 1915, Arch. exp. Path. Pharm., 79, 190.

EXERCISE III.—(OPTIONAL) INSECTICIDES

A method for determining the activity of fluid insecticides is described by Houghton and Hamilton, Mich. Acad. Sci., 1909; for fumigants, by McClintock, Hamilton, and Lowe, Jour. Amer. Publ. H. Assoc., April, 1911.

TECHNICAL REFERENCES

Experiments on Insects.—Fuehner, 50.

Experiments on Invertebrates.—Tigerstedt, 1, 2, 69; Kobert Intox., 1, 154, 166.

Experiments on Plants.—Kobert, Intox., 1, 165; Physiologic Methods, Abderhalden, 8, 222; *Respiration*, Abderhalden, 3, 479; 5, 1271; *Gas and Water Movements*, ibid., 7, 831; *Biochemistry*, ibid., 5, 1263; *Sterilization*, ibid., 6, 137.

PART II

EXPERIMENTS ON ANIMALS

INTRODUCTORY

Objects of the Course.—The experimental course serves to give a direct, observational knowledge of pharmacologic actions, sufficient to permit the student to grasp their essential principles, to obtain a vivid conception of the effects of the more important drugs, and permit him to follow more intelligently the more detailed descriptions of the text-books. Incidentally it also introduces him to the problems of diseased functions (for the effects of drugs are analogous to these) and to their treatment by therapeutic agents.

Observations.—The mechanical performance of the experiments, no matter how carefully they are done, is of relatively small value. At least equally important are accurate observations and interpretation of the results. The conditions in animal experiments are much more complicated than in chemic work. The student must learn to fix his attention on the main phenomenon, without neglecting anything whatsoever. The more functions he can embrace in his observations, the more valuable will be the results and the training. All these observations should be accurately recorded. The student must then ask himself the meaning of these results: What do they really prove? How may they be explained? How could the several possible explanations be confirmed or refuted? What practical significance attaches to these effects? In what diseased conditions could these effects be utilized? How could the toxic effects be treated? etc. The "questions" may serve as a guide, but the more the student thinks along these lines, the greater the value of the course.

Note Taking.—The members of a group or subgroup should collaborate in taking notes, the members alternating as reporters. The notes should be written out legibly while the exercise is being performed, or immediately afterward, the different exercises being kept on separate sheets. These must be handed to the class reporter before leaving the room. The notes should contain a full record of the observations and discuss the conclusions which they justify. The technical methods need only be stated in brief outline, but the doses should always be recorded.

Class Reporters.—Class reporters¹ will be appointed for each day, usually a reporter for each chapter. These are charged with collecting the reports from the individual groups and with combining these into a comprehensive report, aiming to present the essential phenomena, and the conclusions which may be justly drawn from these, without going into extensive details. These reports will be read and discussed at the weekly conferences of the class, and the notes taken on these conferences will serve in place of individual notes.

Questions.—The questions appended to the chapters must be answered by each student individually, in the standard note-book, within a week after the class report has been read.

¹ A list of assignments is given in the Appendix.

Demonstrations, Assignments, and Individual Group Work.—It is desirable that as many of the experiments as possible be performed by the students themselves. The acquirement of the experimental technic is a distinct, although perhaps an incidental, benefit. More important is the fact that many phenomena can be better observed, better grasped, and better understood when they are produced by the student, studied at leisure, and varied at his pleasure, than when observed ready-made, at a distance, and usually only seen for a limited time. Experiments are the more useful and impressive the more they reproduce the method of solving problems by actual investigation.

It should, therefore, be aimed to have each group of students perform for themselves sufficient experiments to illustrate the important principles of pharmacology and the main actions of the most important drugs. However, the experiments performed by different groups may profitably be varied somewhat, so as to illustrate different methods of studying the same phenomenon, and so as to compare and contrast the effects of different drugs. The experiments performed by the student himself will enable him to understand and evaluate these variations when the results are demonstrated to him, or when they are reported in the conferences.

Formal demonstrations, however, are also valuable additions to the individual work. They may be advantageous by presenting experiments which require special apparatus or which are too difficult for the student; they may often save time, and, what is very important, they require a smaller number of animals.

Laboratory Groups.—Partly to save time and animals, and partly to facilitate the most thorough study of the complex phenomena, the students are combined into subgroups (A and B) of three or four men each for the simpler experiments (frogs, intact mammals, etc.), and into full groups of six to eight men for the more complex operative experiments. The members of the groups should alternate in operating, note taking, etc.

CHAPTER XXXII

LOCALIZATION OF ACTIONS; STIMULANTS AND DEPRESSANTS

Explanatory; Stimulation and Depression.—Pharmacologic agents act by increasing or diminishing the normal functions of the tissues. They never create new functions. Exceptions to this rule are few and, indeed, only apparent. They depend on the exaggeration of a function which is normally so slight as to be imperceptible, or which may be latent on account of unsuitable conditions.

An increase of function is called *stimulation*. If it is accompanied by inflammatory phenomena, it becomes an *irritation*, and is necessarily harmful to the tissue. A stimulation may be harmless, although it tends to pass, into fatigue or exhaustion.

A diminution of function is termed *depression*. If the function is entirely abolished, we speak of *paralysis*. This permits of recovery if it involves only one function. If all the functions are paralyzed, we have *death*.

The majority of drugs and poisons produce stimulation at first or in smaller doses; and depression in larger doses. The principal differences are found in the relative degree and duration of the stimulation and de-

pression. A fairly large number of drugs, however, produce depression without preceding stimulation; in a few the stimulation is not followed by depression. In a very few exceptional cases a depression appears to precede a stimulation; but it is likely that this is merely apparent; for instance, it may depend on the involvement of different structures.

The *immediate* and *late effects* of the same drug, and the action of small and large doses are, therefore, often opposed. As a general rule, the large doses produce at first the effects of small doses, even when they have the opposite effect later. It is customary to distinguish these successive actions as *primary* and *secondary* (and sometimes *tertiary*), or, preferably, as *early* and *late effects*.

A critical analysis of the actions of drugs shows them to be very simple in principle: The great majority produce a primary stimulation and second-

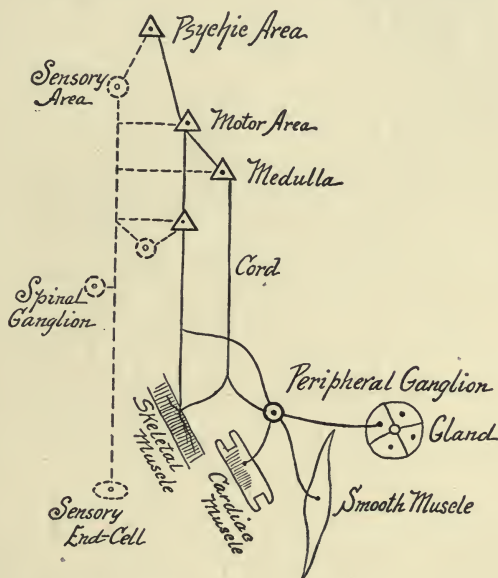


Fig. 7.—Diagram to illustrate possible points of attack of muscle-nerve poisons. The broken line indicates the afferent mechanism; the solid line, the efferent mechanism.

ary depression of most of the structures to which they may be applied. The details, however, present an infinite variety, according to the organs and functions which are most affected.

Most drugs have a *selective action* in this sense. The detailed study of these selective actions constitutes the special aim of *pharmacodynamics*, and is of great importance to the physician.

Principles of Localization of Action.—It is rarely possible to understand the actions of a drug by the observation of the symptoms which it produces. Special experiments are required consisting essentially in the functional isolation of structures which might be involved. The following principles are generally applicable:

The structures which might be involved are considered in the direction of a reflex chain (Fig. 7).

In case of stimulation the links of this chain are successively paralyzed: the site of the stimulation is just central to the point at which paralysis abolished the action. The paralysis is accomplished by section or by drugs.

In case of paralysis, the links of the chain are successively stimulated: the site of the paralysis is just central to the point where stimulation is effective. The stimulation is made electrically or by drugs.

In the actual experiments the structures are not taken in the order named, but according to convenience of technic. It is customary to start with the nerve-trunk and then to work centrally or peripherally as the result may indicate.

TECHNICAL NOTES AND REFERENCES

Frogs.—The common grass (leopard) frog, *Rana viridis* or pipiens, is usually employed in America. "Medium" frogs, of a body length of 2 to 3 inches, answer very well; the larger specimens should be reserved for perfusion experiments. The animals should be kept in a roomy tank, with cold running water and a dry shelf or some stones. A larger size is needed for perfusion experiments.

Administration of Drugs to Frogs.—Solutions are usually injected into the *anterior lymph-sac*. The method of Edmunds and Cushny is recommended: "Lay the animal back downward in the palm of the left hand. Hold one of its forelegs firmly between the thumb and index-finger, and the other foreleg between the middle and ring fingers. Draw its hindlegs downward and hold them against the palmar surface of the hand by means of the little finger.

"Having the drug in the glass injecting pipet, which is held in the right hand, force the animal's mouth open with the point. Pass the pipet into the mouth, avoiding the tongue, which is attached anteriorly, and direct the point toward the floor of the mouth which with a little pressure it will pierce, entering the lymph-sac. As it is pushed down the sac the point can be seen beneath the skin of the abdominal wall. The finger is now removed, and the drug allowed to flow into the sac or, if necessary, blown in."

When *very accurate dosage* is desired, an exact pipet, furnished with a hypodermic needle, may be employed. Ordinarily a pipet graduated by the student with file marks into $\frac{1}{2}$ c.c. will suffice. The quantity injected should lie between 0.25 and $\frac{1}{2}$ c.c.

(*Injection into the Lymph-sac of the Thigh* is described in Chapter XXXVI, Exercise IV.)

Solutions can also be given by the *stomach* through a blunt glass tube passed down the esophagus. Many water-soluble drugs (alkaloidal salts, etc.) are absorbed by the *intact skin*, and may be administered by painting them on the surface of the skin, or by placing the entire animal in a jar containing the solution. *Gases* can be given by placing the animal under an inverted tumbler.

Weighing of Frogs.—The animal is placed in a tared pasteboard box.

Minimum Fatal Dose (M. F. D.).—This is the dose of a drug which is just sufficient to kill a unit weight of an average animal—often in a given time. It is determined by injecting varying doses into a series of weighed animals. Results which differ widely from the average are excluded. The author prefers to take the average between the smallest dose that is fatal and the largest dose that is not fatal. In any case, animals that depart widely from the average, or that show unabsorbed solution, are excluded.

A more accurate relation exists between the dose and the body surface (Dreyer and Walker, 1914, Proc. Roy. Soc., 87 B, 319); but the weight relation suffices for all ordinary purposes. A surface-area formula for man is furnished by Du Bois, 1916, Arch. Int. Med., 17, 863.

Calculation of Doses.—Doses are usually stated as milligrams of drug per kilograms of body weight (mg. \times kg.). The absolute dose is obtained by multiplying this dose by the weight of the animal.

Calculation of Dilutions.—1 c.c. of a 0.1 per cent. solution contains 1 mg. Therefore, to find the most convenient percentage of solution, divide the milligrams of absolute dose by the number of cubic centimeters of solution which you wish to use and multiply the product by 0.1. This gives the percentage. For instance, you wish to inject 90 mg. in such dilution that from 1 to 5 c.c. will be needed. $1 \text{ c.c. would require } \frac{90}{1} \times 0.1 = 9 \text{ per cent. solution; } 5 \text{ c.c. would require } \frac{90}{5} \times 0.1 = 1.8 \text{ per cent. solution.}$ Anything between these limits will answer. Say that a 5 per cent. solution is at hand. Each cubic centimeter of this would equal 50 mg. You wish 90 mg., therefore $\frac{90}{50} = 1.8 \text{ c.c.}$ With a little practice one soon comes to judge the proper dilutions without the necessity of this calculation.

Work out the following problems and see whether the answers are correct: The dog weighs 8 kg. You wish to inject 5 to 10 c.c. of each solution. The dose of (a) = 0.1 gm. \times kg.; (b) = 5 mg. \times kg.; (c) = 0.006 mg. \times kg. What percentage and how much of each solution should be used? Answers: (a) 8 c.c. of 10 per cent. or 1 : 10; (b) 8 c.c. of 0.5 per cent. or 1 : 200; (c) 4.8 c.c. of 0.001 per cent. or 1 : 100,000.

Solution Strengths.—The following tabulation will be found convenient:

100.	mg. = 1 c.c. of 1 : 10 = 10 per cent.
10.	mg. = 1 c.c. of 1 : 100 = 1 per cent.
1.	mg. = 1 c.c. of 1 : 1000 = 0.1 per cent.
0.1	mg. = 1 c.c. of 1 : 10,000 = 0.01 per cent.
0.01	mg. = 1 c.c. of 1 : 100,000 = 0.001 per cent.
0.001	mg. = 1 c.c. of 1 : 1,000,000 = 0.0001 per cent.

Exact Measurement of Solutions.—Quantitative experiments on doses must be made with chemical accuracy. The graduations of syringes are not sufficiently reliable. The solutions must, therefore, be measured with pipets, burets, and cylinders. If a syringe is used, the solution is measured with a pipet into conical glass and drawn from here into the syringe and injected. The glass is then rinsed with a little water or saline, which is also drawn into syringe and injected. (Rosenau describes the inoculation of precise quantities, U. S. Hyg. Lab. Bul. No. 19, 1904.)

Behavior of Frogs.—Kobert Intox., 1, 149.

Motor Stimulation of Frogs, Central and Peripheral.—*Ibid.*, 1, 201.

Motor Paralysis of Frogs.—*Ibid.*, 199.

Convulsants.—*Ibid.*, 220.

Central Nervous System of Cold-blooded Animals.—Tigerstedt, 2,4, 153; Frogs, *ibid.*, 151, 172; successive destruction, *ibid.*, 177.

Turtles.—*Ibid.*, 183; Snakes, *ibid.*, 179.

Removal of brain in frogs and pigeons: Stewart, 961.

Spinal Nerve Roots, Frogs.—Stewart, 957.

Pithing of Frogs.—Frog is held in the left hand and the head bent slightly forward with the thumb. If the finger-nail is passed lightly along the spine a slight depression

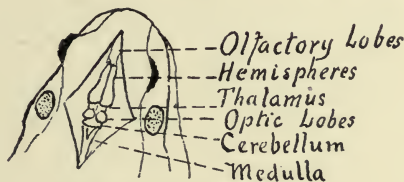


Fig. 8.—Diagram of frog's brain.

will be felt back of the head. A narrow-bladed knife is thrust in here, and the brain or cord can then be destroyed by pushing in a stiff wire. When this is withdrawn the wound should be stopped with a short piece of pointed match to avoid bleeding. A special wire (the thickness of a pencil-lead and $\frac{1}{4}$ inches long) should be reserved for this purpose.

The brain and medulla alone are destroyed when the animal is to be used for the observation of reflexes or circulation. The cord also when the organs (heart or muscle) are to be excised.

To destroy the brain only a line is drawn joining the posterior edge of the tympanic membranes, and the skull opened in front of this line and the brain destroyed (Fig. 8).

Decapitation of Frog.—A blade of a strong pair of scissors is pushed into the mouth, back to the angle of the jaws, and the skull cut away by a single cut, leaving the lower part.

Anesthesia of Frogs.—Frogs may be anesthetized under a tumbler by a pledget of cotton saturated with ether; or, more conveniently, by the injection of 2 c.c. of 10 per cent. urethane into the lymph-sac (Oehrwald, 1911; Skand. Arch. Physiol., 25, 1).

Frog-boards.—For dissections or operations, the pithed frog should be pinned in convenient position on a cork board. Convenient plates of cork $12 \times 4 \times \frac{3}{8}$ inches can be obtained from dealers in shoemakers' supplies. These are cut to a length of 6 inches and may be tacked to small pine boards of about the same size. They may be mounted on a strip of copper that can be grasped in a clamp.

Preparation of the Sciatic Nerve.—The frog is pithed and an incision is made through the skin from the hip to the knee, about the middle of the dorsal surface of the leg. By separating the muscles with the forceps the nerve is seen as a whitish cord at the bottom

of the groove. It may be raised by gently passing a thread under it with a frog-needle.¹ Care must be taken not to injure it by handling. (Fuller description, Fuehner, 84.)

To ligate the leg exclusive of the sciatic nerve, the nerve is prepared as just described, and a stout linen ligature is passed below it and tied firmly around the leg, including all the blood-vessels. The nerve must be protected against drying by covering it with filter-paper soaked in 0.6 per cent. NaCl. (Details, Fuehner, 86.)

Electric Stimulation.—Inductoria.—Induced currents are generally employed for stimulation. The Harvard Inductarium is convenient and suffices for most purposes. The stimulating electrodes are attached to the binding-posts at the end of the metal rods on which the secondary coil slides. The switch between these rods must be open when stimulating.

It is convenient to mount the inductorium on a small board, bearing a primary key connected with the left binding-post. If a dry cell is used, the whole apparatus is conveniently arranged in a small box.

The wires from the battery, etc., for the primary current are attached as follows:

For *tetanizing currents*, to the two outer binding-posts (or to the key and the right post).

For *single shocks*, to the left outer post (or key) and to the middle post.

For *single break shocks*, connect as for single shocks: (1) Close the secondary key; (2) open the primary key; (3) open the secondary key; finally (4) open the primary key, which gives the single break shock. ("Cut-out Keys" are described on p. 793 of the 2d edition of this book; and by Kingsbury & Dresbach, 1910, Quart. Jour. Exp. Physiol., 3, 111; Laidlaw, 1913, Jour. Pharmacol., 4, 461.

The *strength of the secondary current* is regulated by the distance of the secondary from the primary coil, and by revolving the secondary on its axis: the greater the distance

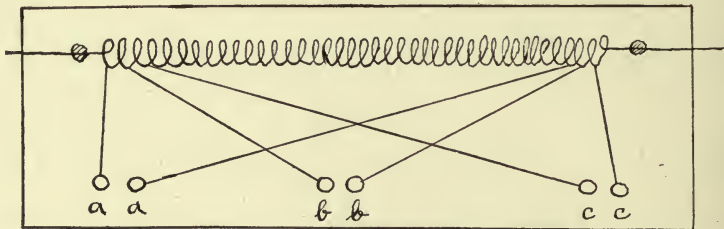


Fig. 9.—Diagram of individual switchboard. The wires leading to the apparatus are attached at *aa*, *bb*, or *cc*.

of the coils, and the greater the angle, the weaker the shocks. (The specific graduation of inductoria is described by Martin, Amer. Jour. Physiol., 1911, 33, 212; 1915, *ibid.*, 36, 223.)

Electrodes.—The ordinary (Harvard, platinum) electrodes are usually employed. For deep-seated nerves the shield electrodes (Harvard) are advantageous. For *direct stimulation of muscle* fine insulated wires are connected with the secondary posts, the other end, freed from insulation, being thrust through and hooked around the muscle.

Non-polarizable and brush electrodes (Mottram, 1915, Jour. Physiol., 49, Proc.) are needed only for special problems.

Source of Current.—Ordinary dry cells may be used; they are conveniently mounted in a little box under the inductorium. Any other type of cells may be employed. If a steady current is required, Daniell cells or a storage battery are essential.

Direct Current.—The direct current is very convenient, especially for class work. It is cut down to the required voltage on the Wheatstone-bridge principle, as described by D. E. Jackson, Jour. Amer. Med. Assoc., 58, 1011, 1912; by v. Hess, 1914, Science, 40, 566; and by Y. Henderson, 1915, *ibid.*, 41, 910.

Any number of coils, etc., may be supplied from a single closed circuit over each table, the circuit passes through a spiral of iron wire ("stove-pipe"), 13 cm. long, wound on a $\frac{1}{4}$ inch rod. The spiral is mounted on an asbestos board and connected with binding-posts, as shown on the diagram (Fig. 9).

Flexible wires, attached to each pair of posts, conduct the current to the coils or other apparatus.

¹ *Frog-needles* are made by heating a stout sewing needle $\frac{1}{4}$ inch from the blunt end until it can be bent at right angles and fixing the point in a convenient wooden handle (perholder).

Perfusion of Frog's Aorta.—Lay the pithed frog on his back, the head toward the operator. With scissors and forceps cut away a flap of skin, from the jaws to the thighs, deflecting it downward. Remove the sternum. Cut away a flap of the abdominal wall and also turn downward. Pin the frog to a board. Tie a small cannula into the peripheral end of the aorta; fill with saline solutions and connect with perfusion bottle.

Observation of Reflex Time.—The frog (usually with brain and medulla pithed) is held with forceps or suspended from a hook passed through the lower jaw, and one or both hind feet immersed in a dish containing 5 per cent. acetic acid or $\frac{1}{2}$ per cent. HCl. The reflex time is the time elapsing between the immersion and the withdrawal of the foot. The average of several observations should be taken, the acid being washed off after each test, and a short interval of rest must be given. (Further discussion, Kobert Intox., I, 191.)

Experiments on Motor Nerves.—Kobert Intox., I, 169; Stewart, 780.

Muscle-nerve Preparation.—The frog is pithed through brain and cord. It is then held up by the legs so that the anterior part of the body falls down. The scissors are thrust through the body a little anterior to the angle and the whole body is cut off. By grasping the skin with a cloth it can be readily removed from the legs. The two legs are then cut apart just in the median line. The iliac bones (the two bones at the sides) are cut away. Each portion is then turned with the posterior surface upward, and the muscles of the thigh are pulled apart with the fingers. The sciatic nerve will be seen lying at the bottom of the groove. It is carefully dissected out with a few cuts of the scissors, from the spinal canal to which it is attached, to the knee. The thigh is then cut off so as to leave a short piece of the femur attached to the knee.†—A blade of the scissors is then thrust under the tendo Achillis, and pushed as far as possible toward the toes. The tendon is then cut off at this point. The tibial bone is also divided close to the knee.—In this way a preparation is formed consisting of a small piece of bone of the spinal column attached to the sciatic nerve, a bit of the femur, the gastrocnemius muscle, and the tendo Achillis. These preparations must be carefully kept from drying by wrapping in filter-paper soaked in normal saline solution.

If the drugs are not to be applied directly to the muscle, the skin may be left on the preparation. If the poison is to be applied only to the nerve, the operation need only to be carried to †.

Gastrocnemius Preparations.—If the muscle alone is to be observed, the preparation of the nerve may be dispensed with. The leg is amputated just above the knee. If the muscle is not to be exposed to the poisons, this preparation may be used as it is. Otherwise the skin may be removed and the muscle prepared as in — to — of the last paragraph.

It is sometimes desirable to obtain a record of muscular contractions while the circulation through the muscle is intact. For this purpose the pithed frog is pinned on the board, dorsal surface up, and a ligature is passed through the tendo Achillis and attached to the lever.

Protection Against Drying.—The muscle and nerve must be carefully protected from desiccation. This is superfluous if the preparation is covered by skin; otherwise, it may be wrapped in filter-paper saturated with normal saline solution. The nerve may be painted with the solution, using a camel's hair brush or swab. If it is necessary to keep the moisture constant, the preparation is covered by a tumbler or bell-jar lined with moist filter-paper. A "moist chamber" is made by the Harvard Apparatus Company.

Direct Application of Drugs to the Muscle or Nerve.—This may be done, according to circumstances, by dipping the part into the solution, or by painting with a camel's hair brush, or by allowing the solution to flow over the part from a pipet. The penetration of solutions into the muscle may be facilitated by scarifying the sheath.

Gases may be applied by placing the preparation, or any part of it, into a tube through which the moist gas is flowing (Harvard gas chamber).

Technical Reference.—Muscle-nerve preparation, Fuehner, 120.

Normal Saline Solution for Frogs.—This is a 0.75 per cent. solution of sodium chlorid.

EXERCISE I.—LOCATION AND TYPE OF CONVULSIONS (FROGS)

(REPORTER I, A)

Explanatory.—This exercise involves the application of the principles just explained. Both strychnin and picrotoxin cause convulsions. The action might conceivably be located in the sensory endings, in the brain, medulla, spinal cord, motor endings, or muscle-fibers. If it is central,

it could be due to direct stimulation or to increased sensibility to reflex impulses.

The student will determine the correct explanation by his experiments.

The type of the convulsions, when once seen, gives a very plain hint of the probable location of the action. The student should tabulate the distinctive differences between strychnin and picrotoxin. This will be facilitated by the use of the following terms:

Opisthotonus: Body arched backward.

Emprosthotonus: Body arched forward.

Clonic Convulsions: Intermittent, jerky.

Tonic or Tetanic Convulsions: Permanent stiffening.

Quite a number of poisons produce the same effects as strychnin; for instance, small doses of caffein; morphin also produces the same action after a time.

Large doses of caffein and veratrin produce effects which resemble those of strychnin superficially. The action of caffein, however, is due to rigor, for it persists after cutting the nerve, and the muscles are inexcitable. Veratrin acts directly on the muscle-fiber, for even the isolated muscle remains contracted for a long time whenever it is stimulated.

Experiment 1. (Demonstration) Strychnin Convulsions.—Inject into the lymph-sac of a frog (Tech. Notes) $\frac{1}{4}$ c.c. of $\frac{1}{10}$ per cent. strychnin. Observe the type of the convulsions carefully (illustrated in Fuehner, 72). Note when they appear; that the legs are extended and the arms flexed; the frog may be held horizontally by the feet. The convulsions intermit, the frog being paralytic between the spasms. The spasms may start with a cry.

The convulsions are typical of spinal stimulation (increased reflex excitability of the spinal cord).

Question.—Describe the effects of strychnin and draw a sketch of the frog in the typical tetanus.

Experiment 2. (Optional) Bio-assay of Strychnin.—Frogs are a more sensitive test for strychnin than are the chemic reactions (Ranke, 1879, Arch. Path. Anal., 75), especially if the solutions are somewhat impure. For the American leopard frog, with injection into the lymph-sac, the tetanic dose of strychnin sulphate is about 0.1 to 0.15 mg. per 100 gm.; the M. F. D. (Tech. Notes) is 0.555 mg. per 100 gm. Decereberated frogs are more sensitive.

Young White Mice give a still more delicate test, those of 4 to 4.5 gm. responding to the hypodermic injection of 0.002 mg. by tetanus within ten minutes; 0.005 mg. being fatal. Tremor of the tail is especially characteristic.

Experiment 3. (Demonstration) Nature of the Stimulus.—Note, on the above frog, that the convulsions appear, as a rule, only when the animal is stimulated.

Note that the following stimuli are effective—touching, jarring the table, sound (clapping hands), electric stimulation of the skin.

Lower a foot of the frog into 5 per cent. acetic acid: the leg is drawn up as in a normal reflex, but there are no convulsions.

Float the frog in a bath of oil: the convulsions are allayed.

Questions.—(a) Does the strychnin stimulate the convulsion centers directly?

(b) Are all varieties of stimuli effective?

Experiment 4. (Optional) Tetanus Threshold.—Pith the brain of a frog (Tech. Notes). Remove the skin from the muscles of one leg. Expose the sciatic nerve (Tech. Notes). Expose the intestines. Set up an induction coil (Tech. Note). Determine the

weakest current which produces *reflex* contraction of the sound leg when applied to the skin of the foreleg and to the exposed muscles, nerve, and intestine. In stimulating the intestines, guard against escape of current to the sciatic plexus.

Strychninize the frog, and when convulsive, test the threshold for tetanus in these various situations. The strongest current will be required for the intestines, the weakest for the nerves of the skin.

Question.—Why are the intestines and muscles less liable to produce convulsions?

Experiment 5. (All A Groups) Location of Strychnin Tetanus.—Inject $\frac{1}{4}$ c.c. of $\frac{1}{10}$ per cent. strychnin into a frog.

(a) Immediately after the convulsions appear, destroy the brain (Tech. Notes): the convulsions continue.

(b) Destroy the medulla in the same frog: the convulsions continue.

(c) Cut all the muscles of one leg through to the femur: this leg ceases to participate in the convulsions.

(d) Destroy the spinal cord: the convulsions cease.

(e) (Optional) Ligate the leg of another frog, exclusive of the sciatic nerve. Inject $\frac{1}{4}$ c.c. of $\frac{1}{10}$ per cent. strychnin below the ligature: no convulsions.

Formulate conclusions justified by each of these experiments as to the site of the strychnin action.

Questions.—(a) Is the strychnin action situated in the brain? (b) In the medulla? (c) In the muscles? (d) In the afferent nerve-endings? (e) Where is its situation?

Experiment 6. (Optional) Location of the Strychnin Action Within the Spinal Cord.—Strychninize the cervical spinal cord, without letting the poison reach the lower portions of the cord:

Insert the lower blade of the scissors in the mouth of a frog, and cut away the entire top of the head, as far back as possible, from the angle of the jaws.

Stop the circulation by opening the frog and excising the heart.

Apply a pledget of cotton soaked in 0.1 per cent. strychnin to the exposed cervical section of the cord.

Test the reflexes by pinching the fore- and hindlegs.

If the experiment is successful, the following results will be obtained:

(a) Pinching the hindlegs causes a normal reflex.

(b) Pinching the forelegs produces convulsions of the entire animal.

Experiment 7. (Demonstration) Inhibitory Influence of Cerebral Lobes on Spinal Convulsions (Acid Fuchsin).—Weigh two frogs (Tech. Note). Use the one (A) as control. From the second (B) remove the anterior half of the brain by cutting with scissors from the angle of the jaws, and just back of the eyes.

Inject into the lymph-sac of each frog acid fuchsin, 0.03 c.c. of 5 per cent. per gram. B will show strychnin-like convulsions within fifteen minutes; in A these will be delayed for one to twenty hours (the further observations may be assigned to one of the A groups). (Barbour and Abel, 1910, Jour. Pharmacol., 2, 167; the action is also much quicker and occurs with much smaller doses, if the heart has been excised; Joseph and Meltzer, 1911, Jour. Pharmacol., 3, 183.)

Questions.—(a) Why does the removal of the brain hasten the onset of the convulsions? (b) What effect has the brain on the reflexes of normal animals?

Experiment 8. (Demonstration) Picrotoxin (Medullary Convulsions).—Inject into the lymph-sac of the frog 1.5 c.c. of 1 : 250 solution of picrotoxin.

(a) Convulsions occur only after a period of depression lasting to half an hour. The animal goes through a regular cycle of motions. (Illustrated in Fuehner, 73.) A characteristic feature is that the legs are abducted in one stage. The animal may turn a somersault. The abdomen may be bloated with air. Between the spasms the animal is depressed.

(b) The convulsions may occur in the absence of stimulation.

(c) Destroy the brain: the convulsions persist.

(d) Destroy the medulla: the convulsions disappear.

(The M. F. D. for medium frogs is about 0.5 mg.)

Questions.—(a) What are the characteristic differences between the strychnin and picrotoxin movements?

(b) Are the picrotoxin convulsions due to direct stimulation, to increased reflex excitability, or both?

(c) Where is the picrotoxin action located?

Experiment 9. (Optional) Other Central Convulsants.—The following may be used on frogs (lymph-sacs):

For Spinal Convulsions.—Hydrastin, 1 to 2 c.c. of 1 : 1000.

For Medullary Convulsions.—Ammonium carbonate, 2.5 c.c. of 1 per cent.; camphor, 1 c.c. of 10 per cent.; phenol, 1 c.c. of 1 per cent.

Experiment 10. (Demonstration) Veratrin (Muscular Spasm).—Inject into a frog 0.5 c.c. of 1 : 10,000 veratrin (= 0.05 mg.). When the effect is fully developed the animal sits normally, but when it jumps the leg remains extended as in tetanus. This relaxes very slowly. If the animal is made to jump repeatedly, its behavior becomes more and more normal, but if it is allowed to rest the stiffness returns.

Pith the brain: the condition remains unchanged.

Divide the tissues of one leg to the bone, and stimulate the muscle with a single shock (Tech. Notes): the muscle still shows prolonged stiffening.

Questions.—(a) How could you distinguish between a strychnin tetanus and a veratrin contracture?

(b) Is the veratrin action central or peripheral? Why?

Experiment 11. (Demonstration) Caffein Convulsions.—Inject a frog with 10 mg. of caffein (1 c.c. of 1 per cent.): marked stiffness and sometimes strychnin-like tetanus.

Experiment 12. (Demonstration) Caffein Rigor.—Pith a frog, and insert a cannula into descending aorta (Tech. Notes). Inject $\frac{1}{2}$ to 1 c.c. of 1 per cent. caffein: immediate rigor. The muscles appear white and hard, do not respond to electric stimulation, and are acid to litmus.

Question.—How could one show that the caffein stiffening is not due to central tetanus?

EXERCISE II.—CENTRAL DEPRESSANTS ON FROGS

(REPORTER II, D)

These produce, successively, quietness; inco-ordination, so that the frog cannot readily turn from its back; muscular relaxation; anesthesia; absence of reflexes.

The paralysis can be shown to be central by stimulating the sciatic nerve: this should evoke a normal contraction of the leg.

Experiment 1. (Group I-B) Morphin (Descending Central Paralysis).—Inject into the lymph-sac of a frog 50 mg. of morphin sulphate ($1\frac{1}{4}$ c.c. of 4 per cent.). Observe the symptoms (which correspond to the ablation of the

central nervous system at successively descending levels). The animal at first becomes quiet, and does not move spontaneously; it sits erect, however, and jumps if stimulated. Place the animal on a small board, and tilt the head-end slowly up: the animal will climb up the board (if observed sufficiently early; later it will not do so). Laid on its back it recovers its normal position. Place the morphin frog and a normal frog in a tumbler filled with water, and invert this over a large jar filled with water (not admitting any air into the tumbler). Both frogs will rise to the top to breathe; but the normal frog, finding no air, will dive down and out of the tumbler; the morphinized frog remains. Remove it from the tumbler and observe that it can swim. Remove from the water. As the action of the poison progresses, the frog will sit more flat. Laid on its back, it makes ineffectual efforts to turn. Still later the frog lies quite flat, makes no effort to turn, and cannot swim. On pinching the toe, the leg still contracts. This shows that the cord and the peripheral sensory and motor nerves are not paralyzed. Lay the frog aside; in the course of some hours or on the next day the animal is found in typical strychnin convulsions. (One of the frogs may receive the morphin several hours beforehand, and the convulsions demonstrated.)

Question.—In what order are the nerve-centers depressed by morphin?

Experiment 2. (Optional) Decerebration on Morphin-tetanus.—Decapitate a frog (Tech. Note). Inject 10 mg. of morphin (1 c.c. of 1 per cent.). Tetanus occurs in one-half to six hours, while a normal frog would require about twenty-four hours (compare with Exercise I, Experiment 7). Cold also favors the onset of the convulsions (Githens, 1912, Proc. Soc. Exp. Biol. Med., 10, 40).

Experiment 3. (Group II-B) Alcohol.—Inject into the lymph-sac of a frog 2 c.c. of 25 per cent. alcohol: paralysis; abolition of reflexes; depressed respiration. Stimulate sciatic nerve (Tech. Note): normal response.

Questions.—(a) Describe the cause of the depression.

(b) Is it central or peripheral?

Experiment 4. (Group III-B) Chloral.—Inject into the lymph-sac of the frog 1 c.c. of 2 per cent. chloral. Observe as for alcohol (Experiment 3).

Experiment 5. (Group IV-B) Ether.—Place a frog under an inverted tumbler containing some cotton saturated with ether: effects as with alcohol (Experiment 3).

Experiment 6. (Group V-B) Magnesium.—Inject into lymph-sac 0.8 c.c. of 25 per cent. $MgSO_4$ for each 10 gm. of frog. Complete anesthesia in an hour. Recovery by next day.

(Optional) *Other Central Depressants* which could be substituted for the above are: Chloroform, 1 c.c. of 20 per cent., in olive oil.

Scopolamin, 1 c.c. of 1 per cent.

Codein, 1 c.c. of 1 per cent.

Thebain, 1 c.c. of 1 per cent. This opium alkaloid acts like strychnin.

Experiment 7. (Optional) Comparative Narcotic Activity.—This is best determined on small fish or tadpoles placed in solutions of different concentration. The "end-point" is complete abolition of all movements except respiration, and recovery in unpoisoned water. Three animals in about 200 c.c. of solution are used for each test. (Details, Fuehner, 52.)

Comparative Analgesic Activity can be determined in man as described by Macht, Herman, and Levy, 1916, Jour. Pharm. Exp. Ther., 8, 1.

Experiment 8. (Demonstration) Anesthesia of "Salted Frog."—Remove the blood from the vessels of a frog by perfusion of the aorta with

oxygenated saline solution: the frog acts as if normal. Expose to ether as in Experiment 5: anesthesia occurs just as in a normal frog.

Question.—Can the action of the anesthetic be attributed to changes in the blood or circulation?

EXERCISE III.—REFLEX TIME

(REPORTER III, A)

This is determined on decapitated (why?) frogs by Tuerck's method (Tech. Notes) by immersing the foot in 0.5 per cent. HCl, and noting the time until it is retracted. The acid is washed off and several determinations are made.

Experiment 1. (Group I-A) Demulcents.—Determine the reflex time of normal decapitated frog, comparing 0.5 per cent. HCl, and 0.5 per cent. HCl containing 15 per cent. of acacia. The reaction is greatly delayed.

Question.—Why does acacia delay the reaction?

Experiment 2. (Group II-A) Alcohol.—Determine the normal reflex time of decapitated frog. Inject into lymph-sac 50 mg. of alcohol (0.5 c.c. of 10 per cent.) and again determine reflex time at intervals.

Question.—What is the effect of alcohol on reflex time?

Experiment 3. (Group III-A) Urethane.—Proceed as in Experiment 2, injecting urethane, 0.2 gm. in 2 c.c.

Experiment 4. (Group IV-A) Morphin.—Proceed as in Experiment 2, injecting 10 mg. of morphin ($\frac{1}{4}$ c.c. of 4 per cent.).

Experiment 5. (Group V-A) Strychnin.—Proceed as in Experiment 2, injecting 0.02 mg. of strychnin (0.2 c.c. of 1 : 10,000).

(Optional) *Other Drugs which May Be Tested on Reflexes:*

Caffein, $\frac{1}{2}$ c.c. of 1 per cent.

Potassium Chlorid, 0.3 c.c. of 5 per cent.

EXERCISE IV.—DEPRESSION OF MOTOR ENDINGS (CURARE ACTION)

(REPORTER IV, D)

Explanatory.—To determine whether a motor paralysis is central or peripheral the sciatic nerve is exposed and stimulated electrically. If there is no response, the paralysis is peripheral. If the muscle contracts, the central seat of the paralysis is located by successive stimulation of the cord and medulla.

A peripheral paralysis may be in the nerve-trunk, the endings, or the muscle-fiber. No drug is known which acts selectively on the motor nerve trunk when applied systemically. The possibility of this action may be excluded by the curare experiments described below. If the motor endings are paralyzed, the muscle will contract if the electrodes are laid directly upon it. This effect is produced most typically by curare; but it is also shared to a minor degree by strychnin, morphin derivatives, coniin, lobelin, camphor, organic ammoniums, magnesium, etc. These drugs, however, have other actions which are much more powerful, and which generally kill the animal in doses much smaller than those required to produce the curare effect. This may, therefore, be very incomplete, or may be demonstrable only by local application to frog's muscles.

Technical References—Tigerstedt, 1, 1, 36; 2, 4, 323; *Preparation of Curarin*, Abderhalden, 2, 942; on small scale, Boehm, 1910, Arch. ges. Physiol., 136, 203; *Curare Paper* (for small doses), Jacoby, 1907, Deut. med. Woch., 1, 1540.

Experiment 1. (Demonstration) Symptoms of Curare Poisoning.—Inject $\frac{1}{2}$ to 1 c.c. of a $\frac{1}{2}$ per cent. solution of curare into the lymph-sac of a frog, repeating the dose every twenty minutes if necessary. Note the general symptoms: the reflexes disappear and the frog shows a general muscular paralysis, but without the preceding cerebral depressions which were observed with morphin. (With some samples of curare, strychnin tetanus precedes the paralysis.)

Experiment 2. (Demonstration) Seat of Curare Action.—When the reflexes have entirely disappeared in the above frog, isolate and stimulate a sciatic nerve. There is no response (or if the poisoning is incomplete, only a slight contraction). The paralysis is, therefore, peripheral to the cord. Apply the electrodes directly to the muscle: there is a strong, normal contraction.

Questions.—(a) Is the curare paralysis central or peripheral? Why?

(b) Does it act on the muscle-fibers? Why?

(c) Where must its action be located? Why?

Experiment 3. (Demonstration) Claude Bernard Experiment.—Take another frog, pith its brain, and ligate one leg, excluding the sciatic nerve. Inject the dose of curare used in (1) into the lymph-sac, and allow it to develop its action. Stimulate the sciatic nerve of both legs: the unligated leg does not respond; the ligated leg contracts. Direct stimulation of the muscle produces contraction in either leg. The ligature which prevented the action of the curare excluded the poison from the nerve-endings, but not from the greater part of the nerve-trunk.

Questions.—Does the curare act on the motor trunk? Why?

Experiment 4. Curare Action on Muscle-nerve Preparation.—The conclusions of Experiments 2 and 3 may be arrived at more simply, and on one animal, as follows: Fit a slide across a small evaporating dish containing the drug dissolved in normal saline; the solution should not reach the slide. Make two muscle-nerve preparations (Tech. Notes) from a fresh frog; determine the threshold current (Tech. Notes) which will give contraction when applied to the nerve and directly to the muscle. Lay the muscle of one preparation on the slide, letting the nerve dip in the solution. Lay the nerve of the other preparation on the slide, letting the muscle lay in the solution. Remove the preparations every five minutes, testing their excitability as described above; replace them, and repeat as often as necessary. Present the results in tabular form:

<i>Distance of coils:</i>	<i>Stimulation of:</i>	<i>Nerve in solution.</i>		<i>Muscle in solution.</i>	
		<i>Muscle.</i>	<i>Nerve.</i>	<i>Muscle.</i>	<i>Nerve.</i>
Before laying in solution.....	
Five minutes.....	
Ten minutes.....	
Etc.....	

If the solution contains a drug with curare action, the nerve which has lain in the solution retains its excitability. The preparation of which the muscle has lain in the solution becomes inexcitable to stimulation by the nerve; the muscle itself retains its excitability.

The following solutions may be used, employing a muscle-nerve preparation from the frogs used in a previous experiment:

(Group I-B): Curare, 1 : 1000 in N. S.	} Use for Experiment 5.
(Group II-B): Nicotin, 1 : 1000 in N. S.	
(Group III-B): Magnesium Sulphate, 5 per cent.	

Questions.—(a) How does this show that the drug paralyzes the motor endings?

(b) Is the action an actual paralysis or a “block”?

Experiment 5. (Groups of Experiment 4) Antagonistic Action of Physostigmin.—Lay the muscle which has been depressed by curare, etc., in physostigmin, 1 : 1000 N. S. Test excitability from time to time: some recovery occurs.

Question.—Does the physostigmin act on the drug, or on the functions?

Experiment 6. (Optional) Antagonism of Physostigmin and Curare in Rabbits.—Anesthetize a rabbit with Paraldehyd, 1 gm. per kilogram, by stomach-tube. Prepare for artificial respiration. Connect the jugular vein with an injection buret.

Inject into the vein physostigmin, 5 mg. per kg. (5 c.c. per kg. of 1 : 1000). This produces fibrillary twitchings.

Divide the sciatic on one side: the twitchings persist. Inject curare, $\frac{2}{3}$ c.c. per kg. of $\frac{1}{2}$ per cent.: the twitchings disappear.

Gradually increase the curare until the respiration stops (being ready for artificial respiration). Note that sciatic stimulation is again ineffective.

Inject physostigmin (several doses if necessary): excitability reappears.

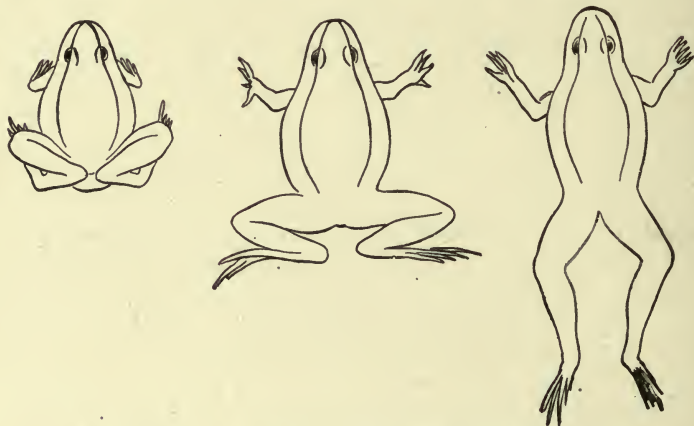


Fig. 10.—Nicotin. Successive positions of frog poisoned with nicotin.¹

Questions.—(a) Is the action of the physostigmin central or peripheral?

(b) How could one treat curare poisoning?

Experiment 7. Direct Paralysis of Muscle.—Use the arrangement of Experiment 4, but employ the following:

(Group IV-B): Saponin, 1 : 1000 N. S.

(Group V-B): Apomorphin, 1 : 1000 N. S.

Question.—What is the site of the depression in these cases?

(Other protoplasmic poisons also paralyze the muscle-cells directly; e. g., cocain or quinin (1 : 1000 to 1 : 100 solutions). Apomorphin and copper salts have the same effect, even when injected systemically.)

Experiment 8. (Demonstration) Systemic Administration of Nicotin.—

(a) Inject into the lymph-sac of a frog 1 mg. (= 1 c.c. of 0.1 per cent.) nicotin. Note that the frog becomes gradually depressed, assuming the characteristic positions illustrated in Fig. 10. (Note the peculiar twitching of the muscles. Divide one sciatic nerve: the twitchings cease. Stimulate

¹ Further illustrations in Fuehner, 75.

the nerve; they reappear. The seat of this action is, therefore, in the muscle or endings, but it can only find expression if the nerve is stimulated from the brain or electrically.)

(b) Make a muscle-nerve preparation from the frog, and test the quantity of current required (*i. e.*, the distance of the coils) to obtain a contraction if the electrodes are applied to the nerve, and if they are placed directly on the muscle; less current is needed on the muscle. Since the reverse is the case in a normal preparation, it is evident that the nicotin must have depressed the nerve-trunk or the endings.

The position of the frog (folding of hindlegs over back) is very characteristic of nicotin, and serves to distinguish it from all related poisons; $\frac{1}{10}$ mg. may be demonstrated in this way.

Experiment 9. (Demonstration) Nicotin in Tobacco Smoke.—Take a small tubulated bell-jar fitted with a doubly perforated stopper. One of the perforations bears a tube reaching just below the stopper. Into the other opening of the cork fit a thistle-tube, which should reach to near the bottom of the jar. Fill the thistle with tobacco. Place a frog under the bell-jar; fix the latter with vaselin on a glass plate. Light the tobacco, and aspirate the smoke into the jar. The frog will show the same symptoms as in nicotin poisoning, since the nicotin is the main active ingredient of tobacco smoke.

Experiment 10. (Optional) Demonstration of Curare Action in Other Drugs.—The following may be used similarly to nicotin:

	In lymph-sac.	Locally (in 0.75 saline).
Camphor.....	0.1 gm.	Saturated
Lobelia.....	2 "	4 per cent.
Conium.....	2 "	4 "
Coniin.....	10 mg.	0.2 "
Lobelin.....	10 "	0.2 "
Magnesium Sulphate.....	1.5 c.c. of 50 per cent. solution.	
Strychnin.....		1 per cent.

EXERCISE V.—PERIPHERAL SENSORY PARALYSIS (LOCAL ANESTHESIA)

(REPORTER V, D)

Explanatory.—Sensory paralysis is evidenced by failure to respond to sensory stimuli (motor paralysis having been excluded by stimulation of the sciatic nerve). Central paralysis is excluded by stimulation of an afferent nerve-trunk. If this proves effective, the sensory paralysis is peripheral. This may involve the nerve-fibers, endings, or sensory end cells. It is not always possible to distinguish absolutely between these. Nerve-trunks are only paralyzed by direct application. As a general rule, this paralyzes both sensory and motor fibers, but the sensory fibers are affected much more readily. It is somewhat easier, however, to demonstrate the paralysis of the motor functions, as in the experiments below.

Sensory depressants are utilized for local anesthesia. General anesthesia may be produced by injecting them into the subdural canal. It must be remembered that they need to be brought into direct contact with the structure to be paralyzed. They are quite inactive on surfaces from which they are not absorbed, such as the intact mammalian skin. On the other hand, they are effective on mucous membranes and the frog's skin. In other situations they are used by hypodermic injection or painted on the nerve, or injected under its sheath. Cocain and its substitutes are the best examples of local anesthetics.

None of the peripheral sensory depressants are sufficiently selective to act from the circulation without producing general intoxication. They are therefore used locally, and in the case of local anesthetics the action is further confined to the place of application by restricting the circulation with a bandage or by suprarenal alkaloid.

Sensory anesthesia may also be produced by very powerful sensory stimulation. Most irritants are succeeded by anesthesia. Aconite and menthol are examples.

Technical References.—*Sensory Paralysis of Frog*, Kobert, *Intox.*, 1, 223; *Eye*, *ibid.*, 1, 215; *Tripolar and other blocks*, Gruber, 1913, *Kansas Univ. Sci. Bul.* 17, Nos. 10 and 11; *Amer. Jour. Physiol.*, 31, 413.

Strength of Local Anesthetics.—The strength of local anesthetics may be tested and compared by the following method:

(a) Rabbit or human cornea (Experiment 2); (b) acid-reflex, frog (Experiment 4); (c) conductivity of the sciatic motor nerve, frog (Experiment 5); (d) conductivity, motor and sensory (reflex), of rabbit's sciatic; (e) infiltration (Experiment 12).

The methods (a) and (b) estimate the anesthetic power for mucous membranes, where absorption is a factor. They give concordant results (Fromherz, *Arch. exp. Path. Pharm.*, 76, 257). The other methods, especially the last, estimate the anesthetic power independent of absorption. (The various methods are described by Fuehner, 165.)

Experiment 1. (Demonstration) Anesthesia of Cornea.—Touch the cornea of a rabbit (or other animal) with a stiff bristle (mounted at right angles on a wooden rod, Fuehner, 168), and note the winking reflex. Apply in one eye a drop of 1 per cent. cocain, in the other a drop of 1 per cent. quinine-urea hydrochlorid. Note that reflex is gradually abolished. This method also shows, by the behavior of the animal, whether the drug is irritant.

Questions.—(a) Which of the two drugs is the more powerful anesthetic? (b) Does either produce irritation?

Experiment 2. (Optional) Exact Estimation of Anesthetic Power on Cornea.—This involves the use of a series of straight hairs of different diameters, 1 or 2 inches long, cemented on the end of small wooden sticks (v. Frey's "Reizhaarmethode").

The sensitiveness of the corneas is tested toward a series of, say, five such hairs, the pain-reaction of the human cornea being the most delicate reaction. A drop of the solution (cocain, 1 per cent.; novocain, 2 per cent., etc.) is then placed in the eye, and the tests with the hair repeated at intervals, observing the time when anesthesia appears and disappears. Both corneas may, of course, be used for different solutions.

The force exerted by the different hairs may be measured by pressing them against a balance and counterbalancing with weights. This weight is divided by the square area of the cross-section of the hair, calculated from its micrometer measurement.

Experiment 3. (All Groups) Anesthetic Action on the Tongue.—This serves as the rough qualitative test.

Place the drug on the tip of tongue (or saturate a small piece of filter-paper with the solution and place on tongue) and test the sensibility to touch.

The (A) groups may use a drop of 1 per cent. cocain; the (B) groups a drop of Tr. Aconite. In the latter the anesthesia is preceded by prickling.

Question.—Would aconite be suitable as an anesthetic for eye-work?

Experiment 4. (B Groups) Anesthetic Action on Frog's Foot.—Test the reflex time (Tech. Note) of a decapitated frog (0.5 per cent. HCl). Dip one foot in 1 per cent. cocain, the other in 1 per cent. solution of some other anesthetic (see below).

Again test the reflex time at intervals of five minutes.

Group I-B—Test 1 per cent. Novocain.

Group II-B—Test 1 per cent. Stovain.

Group III-B—Test 1 per cent. Quinin-urea Hydrochlorid.

Group IV-B—Test 1 per cent. Tropicocain.

Group V-B—Test 1 per cent. Alypin.

Question.—Arrange the drugs in the order of anesthetic efficiency for mucous membranes (the frog's skin is virtually a mucous membrane).

Experiment 5. (A Groups) Paralysis of Nerve-fibers on Direct Application.—Make two muscle-nerve preparations with long nerves (Tech. Notes) from a frog (one used in an earlier experiment). Determine the threshold stimulus of the sciatic. Paint a short stretch of one nerve with 1 per cent. cocain; paint the other with another anesthetic.

Again determine the threshold stimulus in five-minute intervals. When anesthesia is complete, wash the nerve with normal saline and note that its excitability gradually returns. The method really measures the depression of the motor-fibers, which are more resistant than the sensory fibers. However, since the two are generally parallel, it is an admissible, though indirect, measure of anesthetic power. For the comparison the following solutions are to be used:

Group I-A—2 per cent. Hydrocyanic Acid.

Group II-A—1 per cent. Stovain.

Group III-A—1 per cent. Quinin-urea Hydrochlorid.

Group IV-A—25 per cent. Magnesium Sulphate.

Group V-A—Perform Experiment 6.

(Optional) 0.1 per cent. Chloroform.

Question.—Arrange the drugs in order of anesthetic efficiency for neural application.

Experiment 6. (Group V-A) Synergism of Epinephrin and Cocain.—Proceed as in Experiment 5, using four muscle-nerve preparations, as follows (*c* and *d* should be from the same frog):

Lay Nerve (*a*) in 2 per cent. Morphin in N. S.

Lay Nerve (*b*) in 1 : 1000 Epinephrin in N. S.

Lay Nerve (*c*) in 1 : 100 Cocain in N. S.

Lay Nerve (*d*) in 1 : 1000 Epinephrin containing 1 per cent. Cocain in N. S.

Determine the threshold of stimulation in five-minute intervals.

Questions.—(*a*) Does epinephrin hasten or increase the efficiency of cocain?

(*b*) Is epinephrin an anesthetic?

(*c*) How would you explain the synergism?

(*d*) Is morphin a local anesthetic?

(Optional) *Experiments on Other Mixed Local Anesthetics.*—See Zorn, 1913, Zs. exp. Path., 12, 529.

Experiment 7. (Optional) Anesthesia by Gases.—Draw the nerve of a nerve-muscle preparation through a gas chamber, and expose it to the vapors of ether; or to carbon dioxide: stimulation becomes ineffective.

Experiment 8. (Optional) Depression of Conductivity by Ether.—The fact that ether depresses the conductivity as well as the excitability of the nerve can be demonstrated by arranging the sciatic nerve of a muscle-nerve preparation in a small gas chamber on two pairs of electrodes, which are applied to the proximal and distal extremities of the nerve. On conducting ether vapors into the chamber the excitability disappears first at the end of the nerve which is farthest removed from the muscle.

Experiment 9. (Demonstration) Anesthesia of Nerve by Freezing.—Decapitate a pithed frog and trim away the viscera so as to expose the sciatic plexuses. Expose the sciatic nerve of one thigh, without cutting or injuring it, and support it on a match-stick. Lay the frog with the ventral surface upward, arrange electrodes on the plexuses, and see that a weak stimulation is effective (flexing the knee before stimulating). Freeze the exposed sciatic by a spray of ethyl chlorid. The leg will make some spontaneous contraction during the freezing, but in a short time it will cease to respond to the electric stimulation of the plexus, the conductivity of the nerve being paralyzed. Remove the spray and melt the nerve by the heat of the finger: the stimulation again becomes effective after a time.

Experiment 10. (Optional) Anesthesia of Skin by Freezing.—Spray some ethyl chlorid on the back of the hand: this produces pain and then anesthesia.

Experiment 11. (Optional) Intravenous Cocain Anesthesia.—Into an ear vein of a rabbit inject cocain, 10 mg. per kg. (1 c.c. per kg. of 1 per cent.), noting the time of injection.

Observe the motor symptoms: how soon the animal becomes quite paralyzed. Observe also the anesthesia toward pinching or pin pricks. Note the respiration. Is consciousness lost?

Record the time of onset and the duration of the anesthesia.

Question.—What would be the objections to using cocain intravenously on patients?

Experiment 12. (Optional) Infiltration Method of Anesthesia.—(“Quaddel” method of Braun.) Wash the skin of the flexor surface of the forearm with alcohol. With a sharp and strictly sterile hypodermic needle introduced into (not under) the skin, parallel to the surface and just far enough so that cannula-opening is well covered, inject slowly a drop of the sterile solution, so that a small wheal (split-pea size) is formed. Test the sensibility to needle-pricks immediately after injection and in five-minute intervals. The solutions should be made with normal saline and warmed. They may be started with concentration of 0.025 per cent. A number of tests can be made in close succession.

CHAPTER XXXIII

MUSCULAR CONTRACTION: SKELETAL MUSCLE, CILIA

Explanatory.—The actions of drugs on striped muscle are scarcely utilized in therapeutics, but they help to explain the effects on the cardiac muscle, which are very important. They are also of considerable scientific interest. The effects may involve the form of the contraction curve, its height, the rapidity of contraction or of relaxation, the load which the muscle can lift, the total work which it can perform, the promptness of fatigue, the minimal effective stimulus, the latent period, the rate of stimulation required for fatigue, etc. As a general rule, these functions are all affected in the same sense.

The majority of muscle poisons may be arranged in three groups, which are illustrated typically by caffein, quinin, and veratrin.

Caffein increases the activity of the muscle in small doses; larger doses produce phenomena analogous to fatigue. Very large doses throw the muscle into rigor. The methyl-xanthins (caffeine, theobromin, etc.) are the only typical representatives of this group.

Quinin depresses the muscle, and finally paralyzes it, without producing rigor. Only the smallest doses are somewhat stimulant. All protoplasmic poisons and apomorphin and potassium, calcium, and metallic salts produce these effects.

Veratrin causes the muscle to remain contracted for a considerable time, the curve resembling somewhat that of tetanus. It can be distinguished from this by the secondary contraction (see Exercise II, Experiment 2, V); it is, however, an active contraction, for the muscle can sustain a weight. The effect is lessened by all agents which depress the muscle.

In studying the effects of drugs on skeletal muscle, they may either be injected into the lymph-sac or into the aorta; or the muscle may be laid in a solution of the drug in normal saline. Special conditions determine which of these methods is to be preferred. When the muscle is laid in the solution the drug is not always rapidly absorbed. It may therefore happen that one muscle will be scarcely affected by a strong solution, while a weak solution may produce severe effects in another preparation. All the muscular poisons act equally well after curare, showing that their action is indeed exerted directly on the muscle cells.

(Copies of the tracings should be inserted in the note-books.)

Technical References.—*Experiments on Muscle and Nerve.*—Stewart, 780; Tigerstedt, 2,3, 187; Kobert, *Intox.*, 1, 168.

Principles of Registration.—Tigerstedt, 1.4, 51; *Photographic Registration*, *ibid.*, 1.1, 65; 1.4, 25.

Electrophysiology.—*Ibid.*, 2,3, 317; Stewart, 814; *Electrometers and Galvanometers*, Tigerstedt, 2,3, 419; *string*, *ibid.*, 428; *Current of Rest*, Abderhalden, 3, 551; *Action Current as Index of Glandular Activity*, Cannon and Cattell, 1916, *Amer. Jour. Physiol.*, 41, 39.

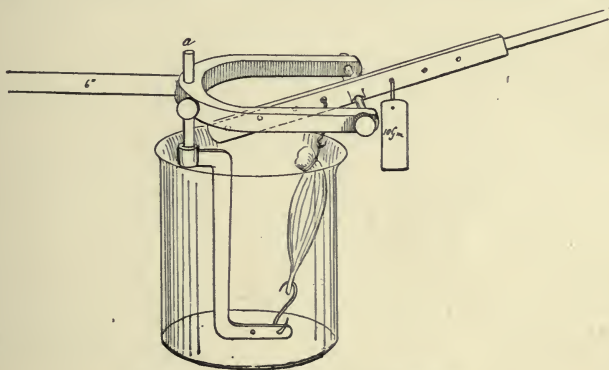


Fig. 11.—Arrangement for muscle tracing.

Technical Notes.—*Tracings from Excised Gastrocnemius Muscle.*—The muscle is attached by hooks or strings, as shown in Fig. 11. The attachment to the lever is best made with a bent pin, so that the point of attachment, and thereby the excursion, can be altered as needed. A weight of about 10 gm. should be suspended on the other limb, about an equal distance from the fulcrum. The nerve may be laid on the electrodes. If the muscle is to be stimulated directly, fine wires, connected with the secondary coil, are thrust directly through the muscle.

Single break shocks are used unless the muscle is to be tetanized. The lever is adjusted at a tangent to the drum until it traces easily when the lever is moved. The writing-point should be bent toward the drum. The fastest speed of the drum is needed to show the form of contraction.

The effect of a solution is tested by placing it in a beaker or test-tube, and raising this so as to immerse the muscle (Fig. 11).

A *time record* may be placed on the tracing by a writing-point attached to a vibrating tuning-fork.

Similar arrangements are described in Heinz, 1, 434.
Muscle Tracings from Intact Frog.—Fuehner, 81.

Muscle Levers.—The substantial pattern shown in Fig. 12 has proved very satisfactory. The muscle is stretched between the arm of the lever and the rod (b) which is set into (a) by a thumb-screw. The levers are prolonged by a narrow strip of aluminum.

L-shaped Levers are required for a horizontal pull.

Other types of levers are described, for instance, in Tigerstedt, 1, 4, 17.

For *writing points* one may use tapering bits of parchment paper 5 cm. long and 1 cm. wide at the base. These are attached to the end of the straw-levers, etc., by sealing wax or colophonium cement. Points of celluloid, aluminum or steel, or the blunt end of a needle, can be similarly used. The end of the writing-point should be bent slightly toward the drum. It should be placed at a tangent, pointing in the direction toward which the drum is moving.

Stands for Supporting Levers, etc.—A rather short stand with heavy semicircular base (Harvard) is best. It is furnished with double clamps ("mouffen").

An *adjustable* stand is very convenient if great accuracy of adjustment is needed. This is secured by a micrometer screw.

Kymographs (Drums).—Movements are registered as "tracings" on cylinders moved by clockwork or motors. The ordinary Harvard kymograph answers for pharmacologic work.

By using two drums (or the device described by McPeck, Jour. Amer. Med. Assoc., 61, 2065, 1913) a longer record can be secured; but this is not necessary if some extra cylinders are smoked in reserve. D. E. Jackson (Jour. Amer. Med. Assoc., 56, 1705, 1911) describes a spinning device for faster speeds. Other types are described in Tigerstedt, 1, 4, 1; Pittenger, 1913, Jour. Amer. Pharm. Assoc., 1498, etc.

Speed of Kymographs.—Three speeds are needed, approximately: 5 to 10 cm. per second for muscle tracings; 10 cm. per minute for details of blood-pressure, etc.; and 2 cm. per minute for prolonged blood-pressure respiration, etc.

A very rapid speed may be secured by raising the drum from the clockwork and spinning it like a top by a weight attached to a cord which is wound about the drum. The tracing should be taken immediately after the weight has fallen.

Tracing Paper.—The cylinder of the drum is covered with paper on which the recording instrument writes. The paper is drawn snugly around the drum, the free edge of the paper being pasted with mucilage on to the first layer. Superfluous paper is trimmed off. The writing may be done with ink from a small glass feeding tube attached to the writing instrument. A more generally useful method, however, is to use a paper with glazed surface and covered with a thin layer of soot, on which the levers, etc., trace.

Dextrin Mucilage (Sykes).—Mix 180 gm. of dextrin with 180 c.c. cold water; add 240 c.c. boiling water and boil five minutes, stirring constantly. Add hot water q. s. 400 c.c. When cold, add 30 c.c. dilute acetic acid, 10 drops phenol, and 30 c.c. of glycerin, previously mixed.

Smoking the Drum.—A uniform layer of soot is deposited on the paper by revolving the drum rapidly in the flame of a fish-tail burner. A stand for supporting the drum while it is being revolved and smoked can easily be constructed from a small box.

A blacker soot may be obtained by passing the gas through a wash-bottle containing a mixture of equal parts of benzin and benzol.

Starting the Tracing.—The tracing is always started where the paper joins; and in detaching it from the drum it is cut along this line.

Abscissa.—It is generally advisable to trace an abscissa on the drum by revolving it against the writing-point before the actual tracing is started. With muscle tracings the abscissa should be at the point of rest; with blood-pressure tracings it should be at the zero level. This abscissa may be used for marking signals and time.

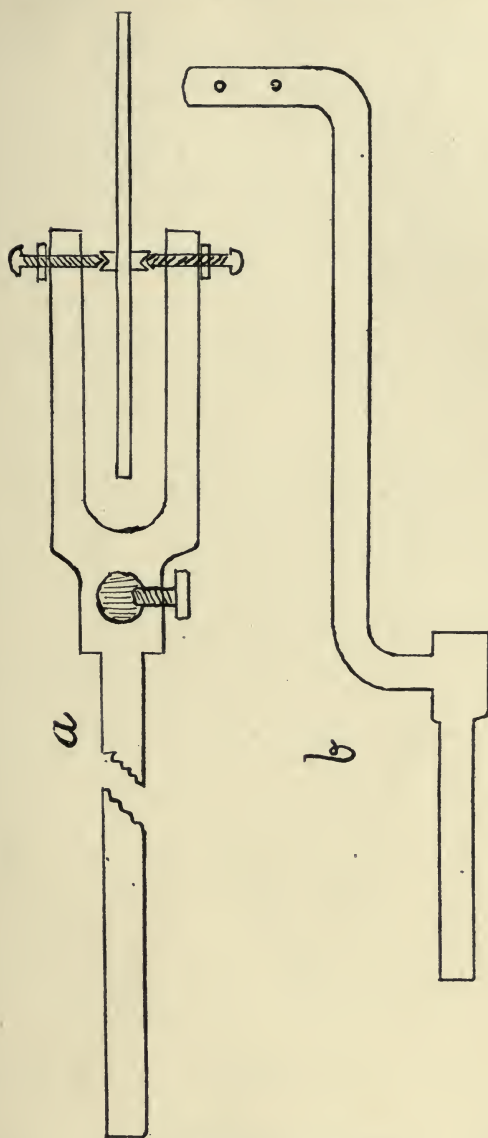


Fig. 12.—Muscle lever, actual size.

Signal Magnet.—The simple Harvard type suffices.

Time Tracings.—For blood-pressure work it suffices to time a single thirty-second period with the signal after the drum is running smoothly. For accurate work, and especially with fast speeds, a clock, such as the Harvard, may be used. This may also be used as signal by connecting an extra key which short-circuits the clock (Fig. 13).

The additional keys may be disposed at various points convenient to the operator. Locke, 1908, *Quart. Jour. Exp. Physiol.*, 1, 359, describes a system of multiple signals with a single lever. Hale, 1916, *Jour. Pharmacol.*, 8, 445, describes a modification of the Harvard time-recording apparatus.

Notes on Tracing.—It is distinctly advantageous to transfer to the tracing all the notes which have been taken during the experiment. This may be done without confusion by numbering the signals to correspond with the notes. The writing on the smoked surface is done with a blunt needle or dry pin, after taking the paper from the drum and before varnishing.

A *marking board* is very helpful to avoid disfiguring the tracing. The tracing is laid on a board over which another board slides on runners.

Varnishing.—The marked tracing is passed through shellac varnish and hung to dry.

Varnish Trough.—Waste of varnish can be prevented by the device shown in Fig. 14. By stepping on the treadle the reservoir is raised so that the varnish flows into the trough. When the treadle is released the reservoir descends and the varnish flows back.

A portable varnish fixture is described by Hoskins, 1916, *Jour. Amer. Med. Assoc.*, 67, 874.

Varnish.—This is made by dissolving orange shellac in 15 parts of alcohol and decanting.

Blue prints of tracings may be made by laying the tracing on a sheet of sensitive blue-print paper, covering with a plate of glass, exposing to sunlight for a day, and washing.

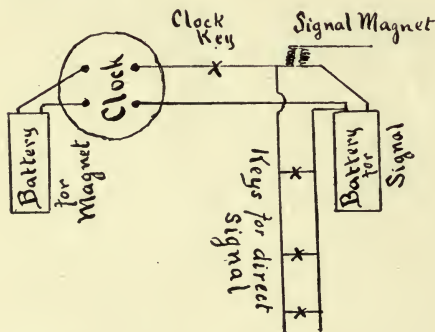


Fig. 13.—Diagram of time and simple signal.

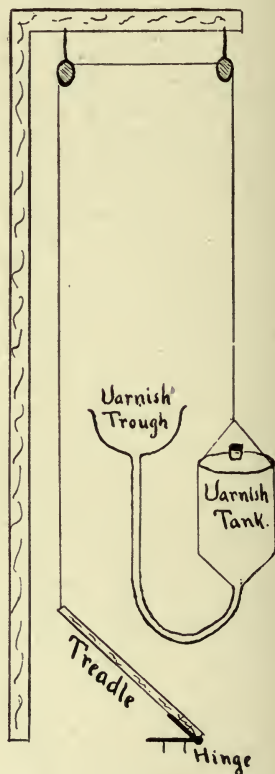


Fig. 14.—Varnish trough.

Lantern-slides of Curves.—Straub, 1913, *Zs. biol. Tech.*, 3, 267.

Demonstration of Tracings.—Tracings may be demonstrated by placing them before a light. An efficient lantern for this is made by a box the size of the tracings, open in front, lined with asbestos, and containing three incandescent lamps. The front of the lantern is closed by two plates of glass, one in front of the other, between which the tracing is slipped. With long paper kymographs the lamps may be hung between the cylinders.

EXERCISE I.—(ALL GROUPS) FORM OF CONTRACTION CURVE

(REPORTER I, B)

Arrange apparatus for muscle-tracing (Tech. Notes) with fastest speed of drum. Arrange induction coil for single break shocks (Tech. Notes, page 136).

Make a muscle preparation of the gastrocnemius, with a bit of femur attached (Tech. Notes, page 137). Tie it on the muscle-lever arranged for immersion in beaker (see Fig. 11). Pass fine wire electrodes from secondary coil into muscle. Immerse muscle in normal saline for five minutes, and make two or three fast tracings of single muscular twitch, single-break shock.

Remove the beaker and replace the saline solution by the drugs named below. Take a very slow tracing, without stimulation, during the course of the immersion, and a fast tracing, with single-shock stimulation, at intervals of five minutes. Where different strengths of solution are to be used they may be changed every five minutes or so. The different groups may do the following experiments:

(Group I) **Caffein**.—Solution in N. S.¹ of 1 : 10,000; then 1 : 1000; then 1 : 100. The more dilute solutions cause a higher contraction, with little change in the form of the curve. Stronger solutions produce a lengthening of the relaxations. The curve then becomes lower, the contraction is slower, and with the strongest solution the muscle does not contract at all. With fairly strong solutions the relaxations may show a series of waves, which are not yet satisfactorily explained.

(Group II) **Theobromin-sodium Salicylate**.—Solutions in N. S. of 1 : 10,000; 1 : 1000; 1 : 100.

(Group III) **Quinin-hydrochlorid**.—Solutions in N. S. of 1 : 10,000; 1 : 1000; 1 : 100. The weakest solutions may increase the height of contraction somewhat; but even fairly weak solutions lower the contraction, and finally paralyze the muscle completely.

(Group IV) **Potassium Chlorid**.—Solutions in N. S. of 1 : 10,000 and 1 : 1000: depression.

(Group V) **Alcohol**.—Solutions in N. S. of 1 : 1000; 1 : 100; 1 : 10: depression. The weakest solution may stimulate somewhat.

(Optional) **Chloroform** or **Ether** may be applied as vapor in a gas-chamber.

QUESTIONS

Enumerate the drugs which increase the height of contraction; those which lower it; and those which have both effects, according to concentration.

EXERCISE II.—(ALL GROUPS) VERATRIN EFFECT

(REPORTER II, B)

Arrange apparatus as for Exercise I, but with fairly slow drum (about 1 inch per minute). Groups I, III, and V: Inject 0.5 c.c. of 1 : 10,000 veratrin into the lymph-sac of a frog. When it shows the typical effects (prolonged extension of legs on jumping) make muscle preparation and take a slow tracing. If the typical action has been reached, the height and rapidity of the

¹ N. S. stands for normal saline solution

contraction is normal, but the relaxation is greatly prolonged. Give the second gastrocnemius to Groups II or IV.

(Optional) The veratrin effect may also be obtained by immersing a thin muscle (the sartorius) in veratrin solution, 1 : 1,000,000 to 1 : 100,000 in N. S.; repeating the stimulation every five minutes until a typical tracing is obtained.

The different groups use the veratrinized muscle for the following experiments:

(Group I) **Incipient Fatigue.**—Stimulate the muscle every five seconds, taking a slow continuous tracing: the relaxation shortens to normal, even before the height of the contraction is lowered.

(Group II) **Temperature.**—Place the muscle in N. S. solution which has been kept on ice. Note the temperature and obtain a tracing. Raise the temperature, immersing the beaker in hot water, so that it takes about five minutes to rise to 10° C. Take another tracing. Continue to raise the temperature, 5 degrees per minute, taking tracings at 15, 20, 25, 30, and 35 degrees. The lower temperatures lessen the contracture; 20 and 30 degrees prolong it; 35 degrees lessen it. (If the veratrin action is only slight, the contracture may appear increased by cold, for this prolongs the relaxation in unpoisoned muscle.)

Heating and Cooling the Muscle.—The muscle may be heated or cooled by laying it in normal saline solution of the required temperature. Better results can be obtained by surrounding the muscle with a box containing water at the proper temperature (Harvard muscle warmer).

(Group III) **Potassium.**—Add KCl 1 c.c. of 1 per cent. per 10 c.c. of veratrin solution, and stimulate at intervals: the relaxation is shortened.

(Group IV) **Ether.**—Add a few drops of ether to the veratrin solution, and stimulate from time to time: the relaxation is shortened.

(Group V) **Secondary Contraction.**—Make a muscle-nerve preparation from a normal frog. Lay the nerve of this on a good veratrin preparation, so that the cut surface lies on the tendon, and the long surface of the belly of the veratrin muscle. The nerve should be raised between the two points of contact by a match-stick. Stimulate the nerve of the veratrin muscle with a single break shock: the current of action will stimulate the normal muscle, so that it will also contract; but the contraction will be short, whereas the contraction of the veratrin muscle is prolonged. This shows that the veratrin contraction is not a tetanus; for if it were, the normal muscle would also remain contracted. Convince yourself of this by stimulating the nerve of the veratrin muscle with the tetanizing current: the normal muscle now remains contracted.

QUESTIONS

- (a) Describe the veratrin effect.
- (b) How may this be antagonized?
- (c) What do these measures have in common?
- (d) How is it proved that the veratrin curve is not a tetanus?

EXERCISE III.—(DEMONSTRATION OR GROUPS I AND II) MAXIMAL LOAD (ISOMETRIC CONTRACTION)

(REPORTER I, A)

Make two muscle preparations. Determine the lifting power as described below. Lay one muscle in N. S., the other in the solutions. De-

termine the maximal load every five minutes, transferring the poisoned muscle to solutions of increasing concentration.

Experiment 1. (Group I): Use Caffein, 1 : 10,000; then 1 : 1000 (in N. S.).

Experiment 2. (Group II): Use Quinin, 1 : 10,000; then 1 : 1000 (in N. S.).

The Lifting Power of a Muscle.—A convenient apparatus for studying this consists in a stiff straight brass wire (4 mm. diameter), about 6 inches long. One end of the wire is securely clamped to a stand; the other is prolonged by a straw, to exaggerate the movement. A stiff iron rod ($\frac{1}{4}$ inch diameter, 6 inches long) is clamped on the same stand, 3 inches above and parallel to the brass wire. The muscle is tied to the two rods so that it may be moved toward or away from the stand. The nearest point to the stand is noted at which stimulation of the muscle causes a perceptible movement of the lever. This will be the nearer, the greater the lifting power of the muscle.

Another method is as follows: The muscle is connected with a Harvard muscle lever, which is supported by the after-load screw. A weight-pan is suspended from the lever at the point where the muscle is attached, and weights are added until the muscle is just unable to move the lever when stimulated.

QUESTION

What are the effects of quinin and of caffein on the lifting power of muscle?

EXERCISE IV.—(DEMONSTRATION) FATIGUE

(REPORTER I, A)

Make two muscle preparations. Immerse one in the poison solution, the other in normal saline for five minutes. Obtain a tetanus tracing (Tech. Notes, Chap. XXXII) first from the poisoned muscle; then just under this on the drum, from the saline muscle. Use the same slow speed of drum, and the same strength of stimulation, for both tracings. Note which fatigues the more quickly.

Experiment 1. (Group III): Use Caffein, 1 : 10,000 in N. S.

Experiment 2. (Group IV): Use Quinin, 1 : 10,000 in N. S.

Experiment 3. (Group V): Use Alcohol, 1 : 100 in N. S.

Ligation and amputation of one leg increases the resistance of the other leg to fatigue (Crider and Robinson, 1916, Amer. Jour. Physiol., 41, 376).

QUESTION

Describe the effects of these drugs on the fatigability of the muscle.

EXERCISE V.—(OPTIONAL)

Action of Drugs on Fatigue in Man (Optional).—This may be studied by the spring ergograph. A normal tracing is taken and this is repeated at half-hour intervals after taking 0.3 gm. of caffein or 20 to 40 c.c. of 20 per cent. alcohol. Some practice is required before reliable results can be obtained.

EXERCISE VI.—(DEMONSTRATION OR ALL GROUPS) OSMOTIC EFFECTS ON MUSCLE AND NERVE

(REPORTER III, B)

Direct contact with water poisons muscle, partly by excessive absorption of water, partly by the withdrawal of salts. These actions are largely

due to osmosis. Strong salt solutions cause irritation and eventually paralysis by withdrawal of water.

Experiment 1. (Group I) Excitability.—Make two muscle-nerve preparations. Use the arrangement described in Chapter XXXII, Exercise IV, Experiment 4. Immerse the nerve of one and the muscle of the other in tap-water, and observe the loss of excitability from time to time.

Note whether there are any muscular twitchings.

The excitability of a muscle or nerve is observed very simply by noticing the greatest distance or angle of the secondary coil which will just give a contraction (single break shocks). Care must be used that the electrodes make good and equal contact.

To compare the effect of a drug on muscle and nerve, two muscle-nerve preparations are made from the same animal. A microscopic slide is placed in an evaporating dish so as to form a bench, and the bottom of the dish is filled with the solution (which should not touch the slide). The two preparations are now arranged so that the nerve of one and the muscle of the other are in the solution, while the muscle of the first and the nerve of the second lie on the bench, *i. e.*, outside of the solution.

Questions.—(a) What are the effects of water on the excitability of muscle and nerve?

(b) Which is more susceptible?

Experiment 2. (Group II) Water Rigor.—Suspend a thin strip of muscle (the sartorius) of frog so that half of it dips into water: this will be seen to become thicker and shorter.

Questions.—(a) Why does the muscle swell in water?

(b) In what way does this affect its functions?

Experiment 3. (Group III) Water Rigor Contracture.—Take slowest speed tracing of gastrocnemius immersed in water (without stimulation). This shows shortening. Determine the weight required to stretch the muscle to its original size.

Questions.—(a) Is the muscle in water-rigor able to sustain a weight?

(b) How does this compare with rigor?

(c) How is the difference explained?

(d) Why does the muscle shorten in swelling?

Experiment 4. (Demonstration) Perfusion with Water.—Decapitate a frog, leaving lower jaw. Divide one sciatic plexus. Insert cannula into descending aorta and wash out the blood with saline. Suspend frog by jaw and attach one foot to light lever.

Perfuse vessels with water: in a short time the muscles will show fibrillary twitchings and these will be succeeded by general convulsions. Eventually there is paralysis.

Questions.—(a) Are the twitchings of central or peripheral origin? Why?

(b) Is the action on the nerve-trunk? (compare Experiment 1).

(c) Is the action probably on the nerve-endings or on the muscle? Why?

(d) How could this be definitely decided?

Experiment 5. (Groups IV and V) Hypertonic Solution on Nerve.—Arrange a muscle-nerve preparation on a lever, writing on a slow drum. Let the nerve dip into 10 per cent. NaCl solution. The muscle will execute a series of contractions, then remain in tetanus, and finally go into paralysis.

Questions.—(a) How does the salt solution act on the nerve?

(b) How could you show that the effect is not due to the NaCl as such, but to the withdrawal of water?

EXERCISE VII.—(ALL GROUPS) RHYTHMIC CONTRACTIONS OF SKELETAL MUSCLE (BARIUM, CALCIUM, DECALCIFICATION)

(REPORTER IV, B)

Disturbance of the ratio of ions about a muscle, as by administration of Barium, by the abstraction of Calcium with citrate or fluorid, etc., brings out the rhythmic functions which are inherent, though latent, even in skeletal muscle. Restoration of the ions again allays these contractions.

Muscles vary greatly in the facility with which rhythmic contractions are induced.

Experiment 1. (Group I) Citrate and Calcium.—(a) Arrange a frog's muscle on a lever, writing on a slow drum. Immerse the muscle in a beaker of 5 per cent. Sodium Citrate. Rhythmic contractions will appear within a few minutes.

(b) Transfer to Calcium Chlorid 1 per cent. in normal saline. When the contractions have ceased, again place in the Citrate and see whether they reappear.

Experiment 2. (Group II) Citrate and Barium.—(a) Same as Experiment 1 (a).

(b) Transfer to 1 per cent. Barium Chlorid in N. S.: the contractions become stronger.

Experiment 3. (Group III) Barium and Calcium.—(a) Take tracing from muscle immersed in 1 per cent. Barium Chlorid in N. S.: rhythmic contractions.

(b) Add an equal volume of 1 per cent. Calcium Chlorid solution: the contractions are not allayed.

Experiment 4. (Group IV) Citrate and Potassium.—(a) Same as Experiment 1 (a).

(b) Transfer to 0.1 per cent. Potassium Chlorid in N. S.: the contractions are allayed.

Experiment 5. (Group V) Fluorid and Calcium.—(a) Same as Experiment 1 (a), but using 0.5 per cent. Sodium Fluorid in place of the Citrate.

(b) Same as in Experiment 1 (b).

QUESTIONS

(a) Why does the cardiac muscle normally contract automatically and rhythmically, and the skeletal muscle only on stimulation and then by a single twitch?

(b) Why are the contractions in citrate and fluorid attributed to the withdrawal of calcium rather than to a direct action of the citrate or fluorid?

(c) How is it shown that the presence of calcium is not sufficient to produce the calcium effects, but that it must be ionized? (Consider Experiment 1.)

(d) Has the production or allayance of rhythmicity any definite relation to the valence of the ions?

(e) Has the action of K in Experiment 4 any relation to the Calcium ions? How could this be shown?

(f) Could the action of calcium be simply that of a depressant? (Consider Experiment 3.)

EXERCISE VIII.—(DEMONSTRATION OR ALL GROUPS) VITALITY OF TISSUES INFLUENCED BY SALTS

(REPORTER V, B)

Excise the hearts of the frogs used in other experiments, and place in watch-glasses with the solutions named below. Note how long they continue to beat:

- (Group I): Ringer's Solution.
- (Group II): Ringer's Solution without Ca.
- (Group III): Ringer's Solution without K.
- (Group IV): Ringer's Solution, triple strength.
- (Group V): Distilled water.

QUESTIONS

- (a) Tabulate the solutions in the order in which the hearts stop.
- (b) Why does the withdrawal of Ca or K injure the heart?
- (c) Why is the heart injured by triple strength Ringer's solution?
- (d) Why is it injured by water?

EXERCISE IX.—(DEMONSTRATION) PROTOPLASMIC DEPRESSANTS

(REPORTER V, B)

Explanatory.—These paralyze nervous and muscular structures, but differ from the muscle-nerve poisons by acting also on monocellular organisms, and often even on ferments. They can be observed conveniently on ciliated cells and on vegetable seeds.

Experiment 1. Paralysis of Cilia.—(a) Cut off the lower jaw of one of the frogs used in a former experiment so as to expose the ciliated mucosa of the pharynx and esophagus. Irrigate with normal saline solution. Determine the time which a small bit of cork requires to travel a certain distance (which may be marked off by pin-pricks). Take a number of observations, keeping the mucous membrane moist. Irrigate with the ether solution, and after a few minutes repeat the observations. It will be found that the ciliary movement is greatly slowed or arrested. If the cilia have not been too profoundly injured they may recover if they are thoroughly washed with normal saline solution.

(b) (Optional) The ether may also be administered in vapor form by supporting the esophagus on a small stand in a tumbler, which contains a little cotton saturated with ether, and which is covered by a glass plate. A recording arrangement (cilioscribe) is described by Dixon and Inchley, 1905, Jour. Physiol., 32, 395.

Experiment 2. (Optional) Germination of Seeds.—"Arrange two 8-ounce wide-mouth bottles with stoppers fitted with glass tubes, letting one tube extend to near the bottom of the bottle. Suspend in each, by means of cotton, a dozen seeds—corn, wheat, clover, beans, etc.—and introduce just enough water to maintain a saturated vapor. Set both bottles in a window. Through one pass *ether vapor*, through the other *air*, twice a day for a week. The seeds in both will swell from the absorption of water, but only the bottle with pure air introduced will grow. Reverse the two. The sprouting grain will have its growth checked and the etherized seeds will begin to grow" (C. W. Greene).

QUESTIONS

- (a) What are the effects of ether on cilia?
- (b) On germination?
- (c) Is the "narcotic" action of ether confined to the nervous system?

EXERCISE X.—(DEMONSTRATION OR ALL GROUPS) ASTRINGENTS

(REPORTER I, F)

Astringents precipitate proteins, thereby diminishing their affinity for water. The tissues, therefore, shrink or contract when exposed to astringents. The astringent power can be demonstrated and compared as in the following experiments.

Technical References.—Dreser, 1908, *Arch. internat. Pharmacod.*, 18, 114; Fuchner, 138; Heinz, 1, 126.

Experiment 1. (Group I) Astringent Action on Lung (Dreser's Method).

—(a) Carefully dissect out a frog lung with its bronchus and insert a small cannula into bronchus (keep lung moist with water). To free end of cannula attach a 2-c.c. pipet divided into $\frac{1}{50}$ c.c.

Insert free end of pipet into a 200-c.c. graduate to a depth corresponding to 50 c.c. and read height (from the cross lines on graduate) that water ascends into the pipet.

Repeat observations at different levels—100, 150, and 200.

Now place lung in a 1 per cent. Tannin solution for two minutes and repeat above observations.

(b) With the other lung make similar observations with water and 1 per cent. Silver Nitrate.

(Group II): Do (a) as above; in (b) use 1 per cent. Zinc Sulphate

(Group III): Do (a) as above; in (b) use Copper Sulphate.

Experiment 2. (Groups IV and V) Astringent Action on Mucous Membrane.—Cut a strip of mucous membrane as long as possible from the mouth of a frog. Attach to a lever and immerse in N. S.; let it trace on a slow drum, first marking a base line. Add to the N. S. the following drugs, and note whether the tracing shows a contraction:

(Group IV): 1 c.c. of 10 per cent. Tannin per 10 c.c. of N. S.

(Group V): 1 drop of Epinephrin, 1 : 1000, per 10 c.c. of N. S.

QUESTIONS

- (a) Arrange the astringents in the order of their efficiency.
- (b) In what conditions would this action be useful?
- (c) Is epinephrin a true astringent?

CHAPTER XXXIV**SMOOTH MUSCLE: INTESTINE, UTERUS, AND ARTERIES**

Explanatory.—The properties of smooth muscle differ in essential respects from those of striped muscle. They are affected in a rather specific manner by the autonomic poisons acting on their muscle substance, on the myoneural junction, or on the ganglion cells. The analysis of these phenomena will be considered later.

The most important smooth muscle systems are those of the gastrointestinal tract—the uterus, the bronchioles, and the arteries.

The phenomena can be most conveniently studied and analyzed on excised mammalian tissues, bathed in warm Locke's fluid, through which a

constant stream of oxygen or air is passed. The muscles may be attached to levers and tracings obtained, just as with skeletal muscle.

Effects of Drugs on Peristalsis.—Drugs which increase peristalsis may be grouped as *cathartics*; those which diminish peristalsis as *antidiarrheica*.

While peristalsis and especially defecation are to some degree controlled by the central nervous system, almost all the drugs which influence them act peripherally. The remedies which are utilized therapeutically to influence peristalsis are mainly direct irritants, chemic or mechanic, or astringents. These act only when they are introduced into the alimentary canal.

Peristalsis may also be influenced by *peripherally acting muscle-nerve poisons*. They are rarely used in therapeutics (except in intestinal paresis), because their effects are not confined to the intestinal tract; but they are of considerable importance in toxicology and pharmacology.

The peristaltic movements are arrested by atropin or epinephrin, stimulated by muscarin, physostigmin, pilocarpin, and nicotin. There is a mutual antagonism between atropin on the one hand and muscarin, pilocarpin, and physostigmin on the other, the effect corresponding to whichever drug is present in excess. Atropin prevents the effects of nicotin, but not vice versa. Barium is active after atropin, but not the reverse.

TECHNICAL NOTES

Decerebration of Mammals.—This is employed when it is desired to exclude disturbing cerebral effects or anesthesia.

Sherrington's *operation for cats* is as follows (Jour. Physiol., 1909, 38, 375; van Leeuwen, 1913, Arch. ges. Physiol., 154, 306; Forbes and Sherrington, Amer. Jour. Physiol., 35, 367):

"The animal (cat) being deeply anesthetized with chloroform, a cannula is inserted into the trachea. Both common carotids are ligated. A transverse incision through the skin is made over the occiput and extended laterally close behind the pinnae. The skin is retracted backward so as to expose the neck muscles at the level of the axis vertebra. The ends of the transverse processes of the atlas are then felt for and a deep incision made through the musculature just behind these processes. The large spinous process of the axis is notched with the bone forceps. A strong thick ligature is passed by a sharp-ended aneurysm needle close under the body of the axis, and is tied tightly in the groove left by the incision behind the transverse processes of the atlas and the notch made in the spinous process of the axis. This compresses the vertebral arteries where they pass from transverse process of axis to transverse process of atlas. A second strong ligature is then looped round the neck at the level of the cricoid, and is so passed as to include the whole neck except the trachea. Decapitation is then performed with an amputating knife passed from the ventral aspect of the neck through the occipito-atlantal space, severing the cord just behind its junction with the bulb. The ligature round the neck is drawn tight at the moment of decapitation. The severed head of the deeply narcotized animal is then destroyed. Hemorrhage is extremely slight. If there is oozing from the vertebral canal it is arrested by raising the neck somewhat above the rest of the carcass. The carcass is placed on a small metal-topped table warmed by an electric lamp below. Artificial respiration is employed to ventilate the lungs, the fresh air supplied from the bellows being warmed by passing through a chamber containing a small electric lamp. The skin-flaps are stitched together, covering the exposed end of the spinal cord and other structures bared by the amputation wound. The carcass will continue for several hours to exhibit good reflexes employing the skeletal muscles, although the arterial blood-pressure is low, often not more than 80 mm. Hg. Reflexes on the arterial blood-pressure are usually obtainable, but are poor. The rectal temperature is fairly well maintained if the table and air from the bellows be suitably warmed; it can easily become too high if the table be overwarmed.

"The execution of the whole procedure occupies about six minutes."

The operation transects the cord about 4 mm. behind the calamus.

It is well to wait for one-half hour to allow the anesthetic to disappear.

The brain may also be cut with a spatula through a trephine opening (Magnus, Arch. ges. Physiol., 130, 254, 1909); spinal animals are not subject to shock by subsequent divi-

sion of the cord at lower levels. The *decerebration of dogs* is described by Sherrington, 1909, Quart. Jour. Physiol., 2, 115.

The central nervous system may also be excluded by the *injection of oil*, etc., into its circulation (Tigerstedt, 3.4, 55); and by the closure of the arteries supplying the brain (Stewart, Guthrie, and Pike, 1906, Jour. Exp. Med., 8, 289); Guthrie, 1911, Zs. biol. Techn., 2, 138); Langley, 1912, Jour. Physiol., 45, 239, secures partial blockage of the fore-brain by the injection of starch suspension into the peripheral end of the right carotid artery.

Decerebration of Rabbits for Survival Experiments.—Morita, 1915, Arch. exp. Path. Pharm., 78, 188.

EXPERIMENTS ON PERISTALSIS IN INTACT ANIMALS

A discussion of the technic is given by R. Magnus, Tigerstedt's Handbuch, 2.2, 115, 1911; also Abderhalden, 6, 604; Kobert, Intox., 1, 250. Additional methods by Hallion and Netter (C. R. Biol., 182 and 254, 1907—balloon method); Alvarez, 1915, myograph, Amer. Jour. Physiol., 37, 267; Joseph and Meltzer, non-anesthetized animals (Soc. Exp. Biol. Med., 7, 95, 1910); Trendelenburg (Zs. Biol., 61, 67, 1913).

Gastric Movements.—Tigerstedt, 2.2, 99.

Hunger Contractions.—Carlson, Amer. Jour. Physiol., 33, 95.

Operations on Digestive Tract.—London in Abderhalden, 3, 76.

Digestive Fistulæ.—Abderhalden, 6, 564; Thiry-Vella, 6, 466.

Digestive Experiments on Animals.—Zunz in Abderhalden, 3, 122.

Collection of Digestive Secretions.—Ibid., 189.

Digestive Tract of Frog.—Kobert, Intox., 1, 187.

Examination of Stomach Contents.—Abderhalden, 8, 44.

Relative Weight of Gastro-intestinal Tract of Rabbits.—Livingston, 1914, Jour. Exp. Med., 19, 339.

Blood-supply of Stomach.—Burton-Opitz, 1910, Arch. ges. Physiol., 135, 205.

EXERCISE I.—(DEMONSTRATION) PERISTALSIS OF EXPOSED INTES-TINES; NICOTIN ON GANGLIA

(REPORTER II, F)

Use a decerebrated rabbit (Tech. Notes). Stretch on board; make small incision in linea alba, and draw forth a loop of small intestine.¹

Experiment 1. Bayliss-Starling Reflex.—Observe that pinching with forceps causes a spreading peristalsis (*mechanical stimulation*); the intestine contracting above the stimulus and relaxing below.

Experiment 2. Local Irritation.—Apply a crystal of salt: spreading stimulation.

Experiment 3.—Apply at another place a few drops of $\frac{1}{10}$ per cent. *physostigmin*: local constriction (stimulation of muscles and endings).

Experiment 4.—Apply at another place a drop of 1 per cent. $BaCl_2$: strong constriction (stimulation of muscle).

Experiment 5.—Apply at another place a drop of $\frac{1}{10}$ per cent. *atropin*: peristalsis ceases.

Experiment 6. Colon Peristalsis.—Pinch the ascending colon, or apply a weak solution of barium chlorid: a tonic contraction ring occurs, and from this starts an ascending peristalsis.

Experiment 7. Nicotin on Ganglia and Nerve-fibers.—Expose the superior cervical ganglion of rabbit. Stimulation causes constriction of the ear vessels and dilatation of the pupil. Paint 1 per cent. nicotin on the nerve below the ganglion. A stimulus applied central to this point is still effective, showing that the nerve-fibers are not paralyzed by the poison.

¹The peristalsis can be evoked, if desired, by placing a bell-jar or celluloid sheet over the intestines and introducing a current of carbon dioxide (Y. Henderson, Amer. Jour. Physiol., 24, 66, 1909). L. Sabbatani makes a window with a watch-glass (Bioph. Centr., 4, 551, 1909). The whole animal may be immersed in bath of warmed saline.

Paint the nicotin on the ganglion. Stimulation of the nerve is now ineffective, showing paralysis of the ganglion.

Experiment 8. Pilocarpin.—Expose the intestines freely. Inject intravenously 3 mg. per kg. of pilocarpin (3 c.c. per kg. of $\frac{1}{10}$ per cent.): the peristalsis is increased (stimulation of the ganglia and muscle). Salivation may be noticed (stimulation of salivary ganglia and endings). The heart is at first slowed, but may be quickened later (peripheral stimulation and depression of vagus).

The heart rate may be demonstrated by a long needle piercing the heart through the chest.

Experiment 9. Pituitary.—Inject intravenously pituitary solution, 0.5 c.c. per kg.: further increase of peristalsis.

Experiment 10. Atropin.—(a) Expose the vagus and determine the smallest stimulus which will just stop the heart. Inject intravenously 1 mg. per kg. of atropin (1 c.c. per kg. of 0.1 per cent.): the peristalsis and salivation cease (paralysis of endings). The heart is quickened, and stimulation of the vagus becomes ineffective (paralysis of vagus endings). The blood-pressure is not much altered; there may be a slight rise. (The rate of the heart will not be changed by the atropin if the pilocarpin paralysis was complete.)

Experiment 11. Barium.—Inject intravenously barium chlorid, 10 mg. (1 c.c. of 1 per cent.) per kg.: strong peristalsis, even after the atropin.

Experiment 12. (Optional) Lead.—Anesthetized cat or rabbit, with window in abdomen. Inject into vein lead acetate, 5 to 8 mg. per kg.: intense peristalsis within five minutes. Lumen nearly obliterated; vessels constricted. The spasm is relieved by intravenous injection of nicotin, atropin, or nitrites (Hirschfelder, 1915, Jour. Amer. Med. Assoc., 65, 516).

QUESTIONS

- (a) Describe the effect of stimulating the intestine by pinching.
- (b) Would this reflex be useful for the propulsion of the contents? Why?
- (c) What is the effect of physostigmin?
- (d) Of barium?
- (e) Of atropin?
- (f) Of pilocarpin?
- (g) Of pituitary?
- (h) Which of the peristaltic stimulants are neutralized by atropin?
- (i) How is it shown that nicotin-paralyzes ganglia?

EXERCISE II.—(OPTIONAL) OBSERVATION OF PERISTALSIS ON UNOPERATED RABBIT

Clip the hair from the abdomen of a rabbit which has been well fed two hours before. Observe the normal peristalsis through the intact abdominal walls. Note the effects of a sudden noise; of ammonia inhalation; of strongly pinching the skin over abdomen; of hypodermic administration of nicotin, 10 mg. per kg.; then of atropin, 5 mg. per kg. (J. Auer, 1907, Proc. Soc. Exp. Biol. Med., 5, 30).

EXERCISE III, A.—(OPTIONAL) ACID ON PYLORIC SPHINCTER

Remove stomach from twenty-four-hour fasting animal; place in warm oxygenated Ringer's solution. Tie cannula in cardia and introduce small quantity of 0.4 c.c. HCl with Congo-red (holding pylorus upward so it will not be touched by acid). Blow into cannula tube until air bubbles through pylorus. Close cannula. When air ceases to escape (*i. e.*, when pylorus is closed), turn stomach gently so acid touches pylorus; this open at once, so that blue fluid gushes out into the Ringer's solution. (Adapted from Cannon, Movements, 106.)

EXERCISE III, B.—(OPTIONAL) ACIDS AND ALKALIES ON TONE OF CARDIAL SPHINCTER OF STOMACH

(See Cannon, "The Mechanical Factors of Digestion," p. 40.)

EXERCISE IV.—(OPTIONAL) BAYLISS-STARLING REFLEX ON EXCISED INTESTINE

Attach a piece of intestine, at each end, to water manometers. Fill with water and suspend in a bath of warm oxygenated Tyrode fluid. On pinching the intestine the manometer at the ascending end should show a temporary fall, the descending end a rise.

EXERCISE V.—(OPTIONAL) EFFECTS OF SMOKING ON HUNGER CONTRACTIONS, HUMAN

See Carlson and Lewis, 1914, Amer. Jour. Physiol., 34, 149.

EXERCISE VI.—(ALL GROUPS) AUTONOMIC POISONS ON RABBIT'S INTESTINE

(REPORTER III, F)

Apparatus for Experiments on Excised Smooth Muscle of Mammals (Intestines, Uterus, Bladder, Arterial Rings).—For each group arrange a large water-bath maintained at 38° to 40° C. In this place a cylinder about 12 cm. high and 3 cm. wide filled with 200 c.c. of warm Tyrode's solution. One or two extra cylinders for changing the solutions may be kept in the bath.

Arrange a muscle lever (Fig. 15) so that tracings may be taken from the intestine, etc., immersed in the solution. When the tissue is in the cylinder a continuous stream of air or oxygen must be bubbled through the solution. The stock of tissues is kept in Tyrode's fluid, to which the blood of the animal is added. For periods longer than an hour the tissues should be preserved in cold Ringer's fluid in an ice-chest.

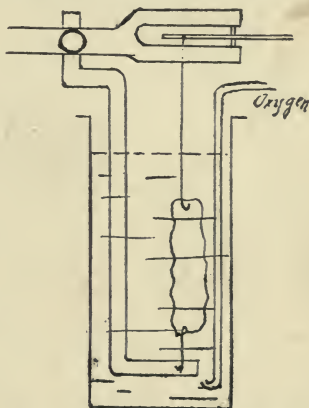


Fig. 15.—Arrangement for excised intestine.

Technical References.—*Experiments on Smooth Muscle.*—Kobert, Intox., 1, 170.

Experiments on Excised Intestine.—Magnus, 1911, Tigerstedt, 2, 2, 141; Stewart, 446; Neukirch, 1912, Arch. ges. Physiol., 147, 153; Gunn and Underhill, 1914, Quart. Jour. Exp. Physiol., 8, 275; Tyrode, 1910, Arch. Internat. Pharmacod., 20, 205; Magnus, 1904, Arch. ges. Physiol., 102, 132 (difference dog and cat).

Tyrode's Solution.—This contains per 1000: NaCl, 8.0; KCl, 0.2; CaCl₂, 0.2; MgCl₂, 0.1; Na₂HPO₄, 0.05; NaHCO₃, 1.0; glucose, 1.0; saturated with oxygen.

Localization of Action of Poisons in Intestines.—Magnus, 1904, Arch. ges. Physiol., 102, 349; 108, 1, 1905; Gunn and Underhill, 1914, Quart. Jour. Exp. Physiol., 8, 275, 296.

Experiments on Excised Ureter.—Macht, 1916, Jour. Pharmacol., 8, 111, 155, 261.

Frog's Esophagus.—Stiles, 1901, Amer. Jour. Physiol., 5, 338; Waddell, 1916, *ibid.*, 41, 529.

Operation.—Kill a rabbit (a female if the uterus experiment is also to be made) by the neck-stroke. Perform artificial respiration, rapidly open the

abdomen, insert a cannula into the abdominal aorta, and bleed dry. An assistant will defibrinate the blood and add it to a liter of cold Tyrode's fluid. (Excised organs preserve their excitability better when kept in the cold; it may be for several days if laid on ice. Their contractions cease in the cold, but resume on heating to body temperature.)

Excise the intestines in mass, also the uterus, and place in the Tyrode blood mixture and pass current of oxygen and air.

From portions of the intestine which show active vermicular movements cut pieces about 5 cm. long, attach to lever, immerse completely in warm Tyrode solution, start air to bubbling, and take a slow tracing. When a normal tracing has been obtained, and while lever is tracing on the drum, add the drugs named below. The quantities are calculated for 200 c.c. of solution. If no response is obtained within a few minutes, further doses of the drug may be added.

(Group I) Epinephrin, 1 drop of 1 : 10,000—inhibition; then Pilocarpin in 1 c.c. of 1 : 1000—contraction; then Atropin, 1 c.c. of 1 : 1000—inhibition; then Barium Chlorid, 2 c.c. of 10 per cent.—contraction.

(Group II) Pituitary Extract, 5 drops—stimulation; then Atropin, 1 c.c. of 1 per cent.—no effect.

(Group III) Pilocarpin, 1 c.c. of 1 per cent.—stimulation; then Atropin, 1 c.c. of 1 per cent.—inhibition.

(Group IV) Nicotin, 1 c.c. of 1 per cent.—stimulation; then Atropin, 1 c.c. of 1 per cent.—inhibition.

(Group V) Barium Chlorid, 5 c.c. of 1 per cent.—stimulation; then Atropin, 1 c.c. of 1 per cent.—no inhibition.

QUESTIONS

- (a) Name the drugs which are stimulant and those which are depressant.
- (b) Describe any differences in the character of the movements.
- (c) Which of the stimulants act more peripheral than atropin?
- (d) Assuming that atropin acts on the myoneural junction, state on which structures the drugs named in (c) probably act.
- (e) Which drugs act more central than atropin?

EXERCISE VII.—SALT ACTIONS ON INTESTINE

(REPORTER IV, F)

Fill the cylinder with warm 0.9 NaCl and immerse fresh piece of intestine, arranged for tracing. Pass air; when slow normal tracing has been taken, draw off the solution and replace by the following¹ (previously warmed in the bath):

- (Group I) Sodium Sulphate, 1.9 per cent.²
- (Group II) Sodium Citrate, 2.7 per cent.²
- (Group III) Magnesium Chlorid, 2.1 per cent.²
- (Group IV) Calcium Chlorid, 0.15 per cent.³ in 0.9 per cent. NaCl.
- (Group V) Sodium Chlorid, 2 per cent.
- (Optional) Water; Urea, 1.9 per cent.²; Cane-sugar, 10 per cent.²; Sodium Phosphate, 2.1 per cent.²; Sod. Acid Phosphate, 2 per cent.²

¹ The percentages refer to the anhydrous salts.

² These solutions have the same freezing-point as 0.9 per cent. NaCl.

³ This corresponds to one-tenth of the isotonic quantity of the salt.

QUESTIONS

- (a) Describe the effects produced by the solutions.
- (b) Which increase the contractions?
- (c) Which diminish the contractions?
- (d) Which increase the tone?
- (e) Which relax it?
- (f) How is the effect of 2 per cent. NaCl explained?
- (g) Does the same explanation hold for the others? Why?
- (h) Sodium sulphate and citrate, as well as magnesium chlorid, are used as cathartics; calcium against diarrhea. Does this agree with their effects on excised intestine?

EXERCISE VIII.—AUTONOMIC DRUGS ON UTERUS

(REPORTER V, F)

Explanatory.—The effects of drugs on the uterus are particularly important in obstetrics and toxicology. The effects differ according to species, pregnancy, etc., but are essentially similar in intact animals and in excised organs. The uterus is stimulated by ergotoxin, histamin, pituitary, etc. Epinephrin contracts the uterus of the rabbit, dog, monkey and human, and pregnant cat; it relaxes that of the guinea-pig and rat, and of the non-pregnant cat.

Experiment.—Cut pieces about 2 cm. long from cornu, arrange on lever, immerse in warm Tyrode fluid; pass air, and take normal tracing. Add the following drugs (per 200 c.c. of Tyrode fluid). If no response is obtained within a few minutes, further doses may be added.

- (Group I) Epinephrin, 1 drop of 1 : 10,000.
- (Group II) Pituitary Extract, 5 drops.
- (Group III) Quinin Hydrochlorid, 2 c.c. of 0.1 per cent.
- (Group IV) Fld. Ext. Ergot, 1 c.c. (or 3 mg. of Ergotoxin).
- (Group V) Tr. Hydrastis, 1 c.c.

TECHNICAL REFERENCES

Uterus in Situ.—Edmunds and Hale, see Exercise VII. Biagi (Centr. Bioch., 4, 762, 1905); Trendelenburg (Zs. Biol., 61, 67, 1913); Ruebsamen (clinical; Muench. med. Woch., 2724, 1913); Pittenger, 71; Barbour, 1915 (Jour. Pharmacol. Exp. Ther., 7, 547).

Uterus, Excised.—Kurdinowski (Arch. Physiol., Suppl., 372, 1904); Kehrer (Arch. exp. Path. Pharm., 58, 366); Prochnow (Arch. internat. Pharmacod., 21, 305, 1911); Pittenger, 73; Gunn (human); Proc. Roy. Soc., 87, 551.

General.—Kobert, Intox., 1, 257, 216; Pittenger, 91.

(Optional) Other drugs which may be used (Kehrer, 1907, Lieb, 1914) are (per 200 c.c.):

1. *Atropin*, 0.1, 1, 10, and 500 mg.
2. *Barium Chlorid*, 60 mg.
3. *Colarnin*, 8 mg.
4. *Histamin*, 0.3 mg.
5. *Hydrastin*, 4 mg.
6. *Hydrastinin*, 8 mg.
7. *Morphin*, 3.3 mg.
8. *Nicotin*, 10 to 20 mg.
9. *Pilocarpin*, 20 to 50 mg.
10. *Physostigmin*, 3 to 20 mg.
11. *Strophanthin*, 0.3 to 3 mg.
12. *Tyramin*, 2.5 mg.

QUESTIONS

- (a) State which of these drugs are depressant, and which stimulant.
- (b) Do the effects of epinephrin and pituitary agree with those on the intestines?

EXERCISE IX.—ARTERIAL RINGS (O. B. MEYER METHOD)

(REPORTER V, F)

Sheep's carotid artery may be obtained from the slaughter-house (it can be kept in Ringer's fluid on ice for several days if necessary). It is cut into rings about 2 mm. wide. One of these is suspended on a "heart-lever" by thread passed through its lumen, so as to record the contraction of the circular muscles. It is immersed in warm Tyrode fluid and the air-current started. The lever is pressed down several times (to overcome the tonus) until it returns to a constant level. It is then made to trace the base line on the drum. The drugs may be added as follows (per 200 c.c.). If no effect is obtained in a few minutes, the dosage may be increased:

(Group I) Epinephrin, 1 drop of 1 : 10,000; then Sod. Nitrite, 1 c.c. of 10 per cent.

(Group II) Sod. Nitrite, 10 per cent., drop by drop; then Epinephrin, 1 drop of 1 : 10,000.

(Group III) Barium Chlorid, 5 c.c. of 1 per cent.; then Sod. Nitrite, 1 c.c. of 10 per cent.

(Group IV) Tr. Digitalis, 1 c.c.; then Sod. Nitrite, 1 c.c. of 10 per cent.

(Group V) Physostigmin, 1 c.c. of 1 per cent.; then Sod. Nitrite, 1 c.c. of 10 per cent.

(Optional) Effect of Calcium on Excitability.—Cow, 1911, Jour. Physiol., 42, 125.

(Optional) Antagonism.—Subject the rings to ergotoxin, 1 : 50,000: moderate but lasting constriction. Change to epinephrin, 1 : 10,000: no effect or slight dilation (Macht, 1915, Jour. Pharm. Exp. Ther., 6, 591).

QUESTIONS

- (a) Which of these drugs produce contraction and which relaxation?
- (b) How does this agree with the effects on the intestines and uterus?
- (c) Does the smooth muscle of different organs necessarily react alike to a given drug?

TECHNICAL REFERENCES

Excised Arteries.—O. B. Meyer, 1906, Zs. Biol., 48, 352; Stewart, 66; Cow, 1911, Jour. Physiol., 42, 125; Barbour, 1912, Arch. exp. Path., 68, 41; Macht, 1915.

Elasticity of Arteries.—Tigerstedt, 2.4, 208.

OTHER SMOOTH MUSCLE

Ureter.—Lucas, 1906, Amer. Jour. Physiol., 17, 392; 1908, *ibid.*, 22, 245.

Bladder.—Stewart, C. C., 1900, *ibid.*, 4, 185; Trendelenburg (intact animals), 1913, Zs. Biol., 61, 67.

Gall-bladder.—Lieb and McWhorter, 1915, Jour. Pharm. Exp. Ther., 7, 83.

Male Genitalia.—Kobert, Intox., 1, 216.

Invertebrates.—Tigerstedt, 1.2, 69; Kobert, Intox., 1, 154, 166.

EXERCISE X.—(OPTIONAL) USE OF SMOOTH MUSCLE IN BIO-ASSAY

The excised uterus and intestines are well adapted for qualitative and quantitative tests, of which the following are important examples:

Experiment 1. Ergot Test on Uterus in Situ.—*Method of Edmunds and Hale*.—A non-pregnant cat is anesthetized with chlorotone, 0.3 to 0.4 gm. per kg. by stomach-tube. Cannulae are placed in jugular vein and trachea. Artificial respiration is started. The animal is submerged in a bath of normal saline of 39° C. The uterus is exposed freely through the linea alba. One horn is freed from its attachments and from the ovary.

Two threads are passed by a needle through the uterus, about 2 cm. apart. These are fastened to a myocardiograph with light lever, put under proper tension, and tracings taken, the drugs being injected into the vein. Injections are repeated every five to ten minutes until same results are obtained as with the standard preparation; 0.2 to 0.3 c.c.

of the Fluidextract of Ergot should cause distinct contractions. The method is tedious and rather uncertain, especially if the uterus is making spontaneous contractions (Edmunds and Roth, 1908).

Experiment 2. Bio-assay of Pituitary.—The excised uterus is now generally used, according to the method of Roth, 1911, *Jour. Pharmacol.*, 5, 559; *Hyg. Bul. No. 100*; U. S. P. IX. Other tests are those of Dale and Laidlaw, 1912 (uterus), *Jour. Pharmacol.*, 4, 75; Hamilton, 1912 (blood-pressure), *Jour. Amer. Pharm. Assoc.*, 1, 1117 (Pittenger, 88). (*Experiments on Pituitary*, Kobert, *Intox.*, 1, 267; *Operations*, Tigerstedt, 2.4, 98; on *Pineal*, *ibid.*, 100.)

Experiment 3. Bio-assay of Epinephrin.—Epinephrin may be tested in several ways, and when its identity is to be established (for instance, in serum) the simultaneous use of several of these methods is indispensable, especially the intestine and uterus (G. N. Stewart, 1911, *Jour. Exp. Med.*, 14, 377). For the quantitative comparison of commercial preparations or gland extracts the pressor effect on mammals (Chapter XLIII) is most convenient; the perfusion of frog legs (Chapter XXXV) and the arterial ring method (this chapter) are also used. The mydriatic test (Chapter XXXVII) is employed for special problems.

The references to these tests are summarized for convenience:

General Discussion.—Crawford, 1907, U. S. Agr. Plant Ind., *Bul. No. 112*.

Mammalian Blood-pressure.—U. S. P. IX; Pittenger, 52; Elliott, 1912, *Jour. Physiol.*, 44, 374.

Presence in Blood.—Stewart, *loc. cit.*; Abderhalden's *Handb.*, 6, 585.

Frog Perfusion.—Fuehner, 140; Trendelenburg, 1910, *Arch. exp. Path.*, 6, 165; 1915, *ibid.*, 79, 154; Tatum, 1912, *Jour. Pharmacol.*, 4, 151.

Ear Perfusion.—Swetschnikow, 1914, *Arch. ges. Physiol.*, 157, 471.

Intestinal Method.—Cannon and La Paz, 1911, *Amer. Jour. Physiol.*, 28, 64; Hoskins, 1911, *Jour. Pharmacol.*, 3, 93.

Uterus.—Stewart, *loc. cit.*

Pupil.—Abderhalden, 5, 112; Meltzer, 1909, *Deut. med. Woch.*, No. 13; Ehrmann, 1905, *Arch. exp. Path.*, 53, 97.

Experiment 4.—Bio-assay of Charcoal Absorption.—Tracings are taken in the usual manner from excised intestine. To 100 c.c. of Ringer's solution add 0.1 c.c. of histamin solution, 1 : 100,000: strong contraction. To another piece, suspended in fresh Ringer's, add up to 10 c.c. of a histamin solution, of the same strength, but which has previously been shaken with blood charcoal, 3 gm. per 100 c.c. of histamin solution. This treated solution, filtered or unfiltered, should be ineffective (Guggenheim, 1915, *Ther. Monatsch.*, 29, 615).

CHAPTER XXXV¹

REACTIONS OF BLOOD-VESSELS (PERFUSION EXPERIMENTS, ETC.)

This subject will be studied in further detail in connection with the blood-pressure experiments. However, the peripheral effects may be shown by perfusion experiments, and some of the general phenomena can be observed on intact animals. The behavior of excised arterial rings was noted in the last chapter.

Technical Notes on Perfusion.—Perfusion, especially of excised organs, is used to study the direct effects of drugs upon their vessels; to produce artificial changes in circulation; to study their work under determinable conditions, etc.

The method consists essentially in circulating liquid through the vessels of an organ under suitable conditions of pressure. The details vary according to the special object and according to the delicacy of the tissue. When dealing with a delicate function it is necessary to take minute precautions as to the composition, oxygenation, temperature, and pressure (preferably

¹ The A and B Groups may alternate Chapters XXXV and XXXVI on successive days.

pulsating) of the perfusion fluid. When investigating the more resistant vascular reactions these complications are superfluous. It suffices to connect the artery of the organ through a cannula with a reservoir of saline solution, placed at a height approximating the normal blood-pressure (Fig. 16). Changes in the caliber of the vessels are denoted by corresponding changes in the vein-flow from the organ. The organ—for instance, the kidney—may also be placed in the oncometer (Sollmann and Hatcher, 1905, *Amer. Jour. Physiol.*, 13, 241).

The *technic for more elaborate perfusion* is discussed by Franz Mueller, 1910, in Abderhalden's *Handb.*, 3, 321; 351; Tigerstedt, 1.4, 51; Kobert, *Intox.*, 1, 171; Friedmann, 1910, *Zbl. Bioch. Bioph.*, 10, 864; Richards and Drinker, 1915, *Jour. Pharm. Exp. Ther.*, 7, 467.

Perfusion for Metabolism.—Abderhalden, 5, 1245.

Perfusion Reservoirs.—"Mercury bulbs" or "aspirator bottles" of 200- to 2000-c.c. capacity may be used. To maintain a constant pressure the upper opening is furnished with a "Mariotte stopper," *i. e.*, a perforated stopper bearing a glass tube which tips to near the bottom of the reservoir.

Constant Pressure.—This is obtained most conveniently by raising the reservoir to the desired level—usually 1 to 1½ meters above the organ—joining it to the arterial cannula by alternate sections of narrow rubber and glass tubing and closed by a pinch-cock. A T-tube, inserted just before the arterial cannula, is convenient for removal of air-bubbles, which must never be allowed to enter the vessels. The T also serves for connection with a second reservoir if the solutions are to be changed.

Warm Perfusion.—A Woulfe bottle filled with the solution and immersed in a water-bath is interposed between the reservoir and the organ. The tube coming from the reservoir tips to the bottom of the bottle; that going to the organ tips about one-third down. The third tubulure bears the thermometer. The organs are supported by cotton, or laid in a bath of warm oil, or suspended in a hot-water funnel (such as is used for filtering gelatin). This allows good drainage.

Rhythmic Pressure.—This is obtained by rhythmically compressing the delivery tube or by opening a side tube (for instance, Gesell, 1914, *Amer. Jour. Physiol.*, 34, 186; for frog, Verworn, *Erregung und Laehmung*, 164).

Oxygen Pressure.—If the solution is to be oxygenated, the oxygen may be used to furnish the pressure, regulating this by a mercury valve (for instance, in the Langendorff heart perfusion apparatus).

Perfusion Stop-cocks.—When a series of fluids are to be alternated several-way stop-cocks may be convenient. They are described by Locke, 1908, *Quart. Jour. Exp. Physiol.*, 1, 370; Macmillan, 1911, *Jour. Physiol. Proc.*, July 22; Mines, 1913, *Jour. Physiol.*, 46, 190.

Measurement of Vein-flow.—The perfusion-flow is estimated most conveniently by the quantity of fluid flowing from the vein. If the changes are relatively slow, it suffices to insert an elbow cannula into the vein and collect the fluid, determining either the quantity collected in a given time or the time required to collect a given volume.

If the changes are fairly rapid, the flow may be measured by a *dipping bucket* (W. R. Williams, 1910, *Jour. Pharmacol.*, 1, 457; Condon, 1913, *Proc. Physiol. Soc.*, *Jour. Physiol.*, 46); or a *Ludwig stromuhr* (Sollmann and Pilcher, 1910, *Amer. Jour. Physiol.*, 26, 236). Other methods, used especially for *vein-flow in intact animals*, are those of Barcroft and Brodie, 1905, *Jour. Physiol.*, 33, 53 (rise of tambour); Wiggers, 1908, *Amer. Jour. Physiol.*, 23, 23 (scale pan); Brodie and Vogt, 1910, *Jour. Physiol.*, 40, 135 (oncometer); Brodie and Russel, 1905, *Jour. Physiol.*, 32; Ishikawa and Starling, *Jour. Physiol.*, 45, 164; Burton-Opitz, 1908, *Arch. ges. Physiol.*, 121, 150 (vein stromuhr); W. Trendelenburg, 1914, *Zs. Biol.*, 65, 13; see also Tigerstedt, 2.4, 259; Heinz, 2, 145; Kobert, *Intox.*, 1, 233.

Drop Recorders.—These are used when the flow of liquid is slow. A simple type is shown in Fig. 17. In demonstrations a small electric lamp may be inserted in the circuit. Another simple type is described by Fuehner, *Nachweiss*, p. 143. See also Abderhalden's *Handb.*, 5, 109; Macmillan, 1913, *Quart. Jour. Exp. Physiol.*, 6, 109.

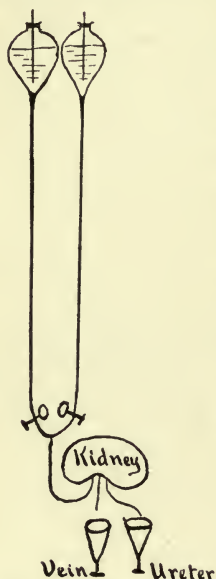


Fig. 16.—Diagram of kidney perfusion.

Oncometers (Plethysmographs).—These are instruments for observing and measuring changes in the volume of an organ. A very simple form may be made of a conveniently shaped tin box, which has two openings, one for the vessels of the organ, another for the tube of the recording apparatus. This consists of an elongated thin rubber bag (such as is used in toy balloons), connected with a water manometer. The bag is filled with water, connected with the manometer, and folded about the organ within the box. When the latter is closed, any change in the volume of the organ is communicated through the bag to the manometer. It may be recorded by connecting the free limb of the manometer with a Brodie bellows or piston-recorder.

More elaborate forms are described by Roy, Schaefer's Textbook, 1, 643; Schaefer and Moore, 1896, Jour. Physiol., 20, 1 (gutta serena); Edmunds, Jour. Physiol., 22, 380 (intestines, plaster); 1913, Zs. Immun., 17, 119; Jour. Pharmacol., 5, 520, 1914; Jour. Pharmacol., 6, 589, 1915 (liver); Cloetta, 1910, Arch. exp. Path., 63, 147 (lung); also Tigerstedt, 2, 4, 272; Heinz, 2, 154. *Plethysmograph* for extremities and *Recording Devices*, see Exercise VIII.

Preparation of the Organs for Perfusion.—The animal is usually bled. (If the perfusion is to be made with diluted blood, a liter or two of Locke's solution is run into the femoral vein and the animal is again bled.) The bloods are defibrinated by whipping, strained through cloth, and poured into the reservoir. The organ is exposed, a cannula

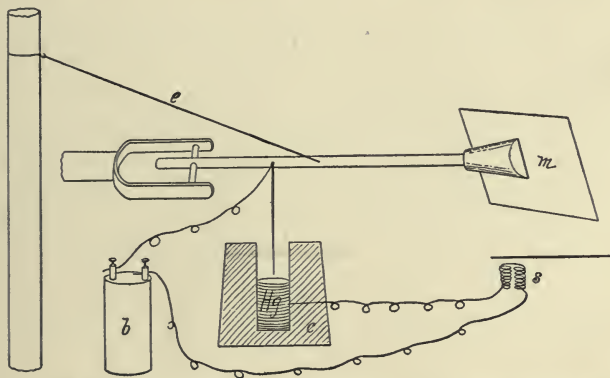


Fig. 17.—Drop marker: A small mica slide (*m*) is fixed at the end of the muscle-lever by means of a small cork. The mica slopes downward. The lever is kept horizontal by a long band of thin elastic rubber (*e*), so that a drop falling on *m* will cause the pin *p* to dip into the mercury in the hollow cork *c*, closing the circuit with the battery *b*, and moving the magnet *s*, which writes on the drum. The outflow tube is placed at least a foot above the mica slide.

is tied in its artery, and connected with the reservoir. The vessels are well flushed (to prevent clotting). The vein cannula is now tied in. All other vessels are tied and the organ is removed. To avoid drying it may be covered with a muscle-skin-flap from the abdomen of the dead animal.

Cannulae.—A plentiful assortment of different sizes and forms should be on hand. They are best made from glass tubing. The edges should not be sharp; they may be rounded in the flame or on a sandstone.

Vessel Cannulae.—Fig. 18, *a* to *d*, shows the shape and the most useful sizes: *a* is for use in the frog's heart; *b* for rabbit's carotid or dog's femoral artery; *c* for dog's carotid artery or femoral vein; *d* for dog's external jugular. A still smaller size is needed for glandular ducts.

These cannulae are made by heating the proper size of tubing in a large blow-pipe flame and drawing it out in the form of Fig. 19. This is allowed to cool and cut at *a*. The pieces are then heated with a very small pointed flame at *f*, so as to make the shoulder. The ends are cut off as obliquely as possible by scratching with a triangular file, ground to the proper form on a grindstone, and rounded in the flame. A good cannula should have the end sufficiently large so that it will not slip when tied into the vessels, but no larger.

(In heating glass, it should be constantly rotated in the flame; it is well to push it

together *very* gently while heating. It should always be removed from the flame before drawing.)

Tracheal Cannulae.—These are of the form shown in Fig. 18, *e*.

One end is best made somewhat smaller than the other, so that the same cannula may serve for somewhat different sizes of trachea. Tubing 5 and 8 (Fig. 20) is most useful for rabbits; 9, 10, and 12, for dogs. The Harvard metal cannulae serve excellently.

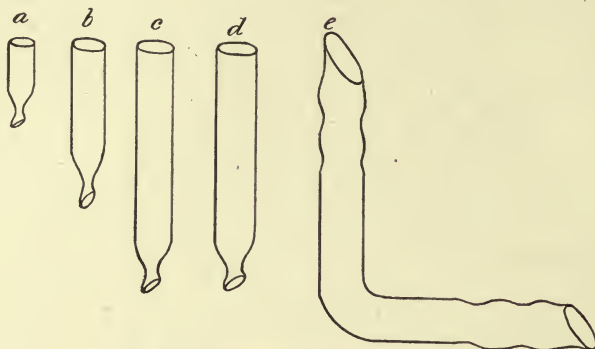


Fig. 18.—Cannulae for vessels and trachea.

Aortic and Bladder Cannula.—This is made of the form and size of Fig. 21. The rings are made by heating a narrow zone of the tube in a small flame, and pushing the glass together. When used on the bladder, this cannula is tied in the neck. Another bladder cannula, used especially in rabbits, consists of a short thistle tube (Fig. 22). The bladder is cut open and tied as a drum-membrane over the mouth of the cannula, the ureters being left free and opening into the cannula.

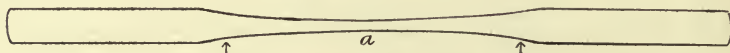


Fig. 19.—Tube drawn for cannulae or pipets.

Ureter cannulae are given the form shown in Fig. 23. This is the proper size for dogs. A smaller tube is required for rabbits. The narrow tubing is obtained by using the portion between the arrows in Fig. 19, making this somewhat longer.

Insertion of Cannulae Into Vessels.—The vessel is exposed and cleared of all fascia for the space of 1 inch, if possible. A bulldog forceps¹ (Fig. 24) is then applied to the end of the vessel toward which the cannula will point. A ligature is passed by forceps or aneurysm needle around the vessel near the clamp, and tied into a loose slip-knot. The vessel is then allowed to fill with blood, and another ligature tied securely as far away

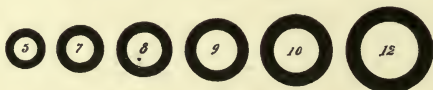


Fig. 20.—Sizes of glass tubing

from the clamp as possible. The vessel is now lifted by the end of the second ligature and laid on the left index-finger. An incision is made with small curved scissors near the distal ligature, about two-thirds through the vessel, the moistened point of the cannula is pushed in, and the loose ligature is tied securely around the neck. The ends of the ligatures are now cut off. The largest cannula should be chosen which will fit the vessel without force. The cannula is turned within the vessel so that kinking will not close the opening of the cannula.

¹ When buying these clamps one should take care that the jaws touch along their entire surface.

The whole procedure is quite easy when the vessels are strong. Delicate vessels should be well distended, and all twisting must be avoided. It may be necessary to hold the vessel open with very fine-pointed forceps. The manipulations must be made very delicately.

Ligatures.—It is a mistake to use ligatures which are too thick. The following are useful sizes: No. 50 linen thread or buttonhole-twist silk for vessels; cotton wrapping twine for trachea, bladder, etc. They should be cut to a length of about 6 inches. (This may be done in mass by winding the string around the palm of the hand.)



Fig. 21.—Aortic and bladder cannula. Actual size.



Fig. 22.—Bladder cannula.

A ligature should be tied as securely as its strength will allow. A little practice will show its limitations. More force can be exerted if the pull is made very near to the knot. A plain double knot is best for small vessels; the bulky surgeon's knot should be confined to larger structures, such as the trachea or aorta.

Ureter cannulae are introduced in the same manner as described for the vessels, except that the ureter need not be clamped.

The same general method is also used for inserting the **tracheal cannula**. The trachea is exposed, cleaned, two ligatures are placed 1 or 2 inches apart, and three or four rings of cartilage are divided with the knife by a straight or V-shaped incision.

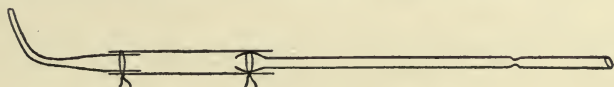


Fig. 23.—Ureter cannula. Actual size.

Perfusion of Brain.—Dixon and Halliburton, 1910, *Quart. Jour. Exp. Physiol.*, 3, 315; *in situ*, E. D. Brown, 1916, *Jour. Pharmacol.*, 8, 185.

Perfusion of Liver.—Baglioni, Abderhalden, 3, 364; Macleod and Pearce, 1914, *Amer. Jour. Physiol.*, 35, 87; *Frog*, Morita, 1915, *Arch. exp. Path. Pharm.*, 78, 232.

Perfusion of Lung.—Baehr and Pick, 1913, *Arch. Exp. Path.*, 74, 42; Tigerstedt, 24, 296; Magnus and Sorgdrager, 1914, *Arch. ges. Physiol.*, 155, 192; Modrakowski, *ibid.*, 158, 509.

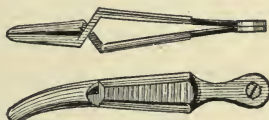


Fig. 24.—Bulldog clamps.

Coronary Perfusion.—Morawitz and Zahn, 1914, *Deut. Arch. Klin. Med.*, 116, 364.

Heart-lung-kidney Preparation.—Bainbridge and Evans, 1914, *Jour. Physiol.*, 48, 278.

Splanchnic Vessels, Frog.—Froehlich and Morita, 1915, *Arch. exp. Path. Pharm.*, 78, 277.

Perfusion Fluids.—The plain *Normal Saline Solutions* contain NaCl sufficient to render them isotonic with the blood-serum (0.75 per cent. for frogs; 0.7 per cent. for mammals). They suffice for injections into living animals, but not for excised tissues or

perfusions. For these it is necessary to use more complex fluids. The more important of these are shown in the following table (also Tigerstedt, 2.4, 170).

TABLE OF COMMONLY USED BALANCED SOLUTIONS

Author.	Adapted to—	PERCENTAGES:				
		NaCl.	KCl.	CaCl ₂ .	NaHCO ₃ .	Other ingredients.
Ringer.	Frog's heart.	0.6	0.0075	0.01 (dried). 0.026 (crystals).	0.01	
Howell.	Frog's heart.	0.7	0.03	0.025 (crystals).	0.003	
Clark.	Frog's heart.	0.7	*	0.012 (dried).	0.02	
Goethlin.	Frog's heart.	0.65	0.01	0.0065 (dried).	0.01	(Na ₂ HPO ₄ , 0.0009 NaH ₂ PO ₄ , 0.0008 Dextrose, 0.1
Locke.	Mammalian heart.	0.92	0.042	0.024 (crystals).	0.015	
Rusch.	Mammalian heart.	0.8	0.0075;	0.01 (dried).	0.01	
Tyrode.	Mammalian intestine.	0.8	0.02	0.02 (crystals).	0.1	(MgCl ₂ , 0.01 Na ₂ HPO ₄ , 0.005 Glucose, 0.1 MgSO ₄ , 0.03 Na ₂ HPO ₄ , 0.05 Glucose, 0.1 MgCl ₂ , 0.025 Na ₂ HPO ₄ , 0.0126 Glucose, 0.15
Hedon and Fleig.	Mammals.	0.6	0.03	0.01 (dried).	0.15	
Adler.	Mammals.	0.59	0.04	0.04 (crystals).	0.351	

* The lower K content gives a more rapid heart-rate.

Note 1.—In making solutions containing NaHCO₃, this must be completely dissolved before the CaCl₂ is added.

Note 2.—Other solutions are described in "Digests of Comments on Pharmacopoeia," 1911, p. 611.

Stock Solutions.—As the perfusion fluids are often used in considerable quantities it is convenient to prepare them as concentrated stock solutions twenty times the original strength. The concentrated calcium solutions should be kept separate, and added after the other ingredients are diluted.

Solutions of Salts giving the same freezing-point as 1 per cent. Sodium Chlorid (1 gm. of NaCl added to 100 c.c. of distilled water; $\Delta = 0.589$; molecular concentration = 0.316).

All the salts are to be weighed in grams and made up to 1 liter with distilled water. They should first be *dried* to constant weight at 110° C. unless otherwise stated. They must always be controlled by actual freezing-point determination.

Checked by the Author:

BaCl ₂	25.62
CaCl ₂	16.33
HCl.....	10 c.c. = 15.8 c.c. n/10 NaOH
LiCl.....	7.26
MgCl ₂	21.15
Na Acetate.....	12.75
NaHCO ₃	9.66 (Do not dry)
NaClO ₃	17.95
Na Citrate.....	27.37 (47.33 to 75.73 crystals)
NaNO ₃	15.35
Na Oxalate.....	23.00
Na ₂ HPO ₄	21.00
Na ₂ SO ₄	21.00 (47.73 crystals)

Deduced from Published Tables:

Alcohol.....	14.50
plus 1 liter	
Cane Sugar.....	108.82
plus 1 liter	
Glucose.....	56.74
plus 1 liter	
Urea.....	18.94
plus 1 liter	
MgSO ₄	35.37
Na ₂ CO ₃	14.54
NaOH.....	7.00

Deduced by Analogy:

NH ₄ Cl.....	9.13
NaBr.....	17.46
NaI.....	25.42
NaCNS.....	14.24
NaF.....	7.21

EXERCISE I.—(DEMONSTRATION) NICOTIN ON EAR VESSELS. (VASO-DILATION FROM DEPRESSION OF VASOCONSTRICTOR GANGLIA. VASOCONSTRICTION THROUGH REFLEX STIMULATION.)

(REPORTER I, C)

Inject a white rabbit with 10 mg. per kg. of nicotin (1 c.c. of 1 per cent. per kg.): in about ten minutes the ear vessels are seen to dilate. (Depression of the sympathetic ganglia.) Apply reflex stimulation (blowing on the rabbit): the vessels constrict at once; after a short time they dilate again, and the experiment may be repeated indefinitely. (The small dose of nicotin used in this experiment produces a depression of the ganglia sufficient to block the weak tonic vasoconstrictor impulses which pass normally to the muscle; but it is not sufficient to block stronger impulses, as those due to reflex stimulation. Larger doses of nicotin block these impulses also.)

The *general effect* of nicotin may also be observed on this animal. The reflex excitability is first increased, then the animal shows a condition of partial paralysis, with convulsions on stimulation. There may be nausea. The pupils are variable.

Questions.—(a) What vasomotor changes are produced by nicotin?
(b) Describe the symptoms of nicotin poisoning.

EXERCISE II.—(DEMONSTRATION) ERGOT ON COMB OF ROOSTER

(REPORTER I, C)

Administer to a rooster 5 gm. of powdered ergot (rolled into a cartridge with tissue paper) by mouth, or 5 c.c. of fluidextract hypodermically. Within an hour the tips of the comb and wattles will become cool and blacken. This may persist for several days and may pass into dry gangrene of the affected parts. The result is due either to a persistent vasoconstriction resulting from a direct action on the arterial muscle, or to some change in the endothelium. (The experiment is often unsuccessful if the ergot has become inactive, or if the animal is not very susceptible.)

EXERCISE III.—(OPTIONAL) ASSAY OF ERGOT ON ROOSTER-COMB

This is probably the most reliable test for the activity of ergot. The official method is described in the U. S. P. (also Pittenger, 69).

EXERCISE IV, A.—(DEMONSTRATION) PERFUSION OF FROG'S VESSELS (LEWEN-TRENDELENBURG METHOD)

(REPORTER I, C)

The method consists in the perfusion of the legs of the pithed frog through the abdominal aorta from a Mariotte bottle. The outflow from the abdominal vein is recorded by a drop-counter. The drug is injected with a syringe into the tubing leading to the aorta. The flow is slowed by constrictor drugs, and vice versa. The details are as follows:

Decapitate a large frog and pith the spinal cord. A strip of skin, 2 cm. wide, is cut away from the chest and abdomen. The sternum is removed. The large median abdominal vein is divided just below the sternum, and a strip of the abdominal wall with this vein is cut and reflected toward the legs. The *venae renales advehentes*, going from

the thigh toward the kidneys, are surrounded by ligatures and tied. The abdominal organs are then removed, avoiding injury to the aorta or abdominal vein.

The frog is now fixed to a cork-board (Fig. 25). A very fine, long-pointed cannula is tied into the aorta, so that its point is just above the bifurcation. The cannula was previously connected by rubber tubing (about 40 cm. long) with a 250-cm. Mariotte bottle (Tech. Notes), filled with Ringer's fluid. The connecting tube bears a screw-clamp, which is opened slightly during the introduction. Air bubbles must be rigorously excluded. The tubing is fastened to the board.

When the fluid drops from the abdominal vein a thin glass tube, about 1 mm. in diameter and about 6 cm. long, is tied into the vein. The free end of the tube is bent to facilitate dropping and raised somewhat over the board by a small cork.

The Mariotte bottle (Fig. 26, *MF*) is now adjusted at such a level (perhaps 15 cm. above the frog) that the vein delivers 30 to 40 drops per minute. A drop-counter (Fig. 17, p. 169, is arranged under the drops. The marker is adjusted on a drum, together with a time-marker tracing second.

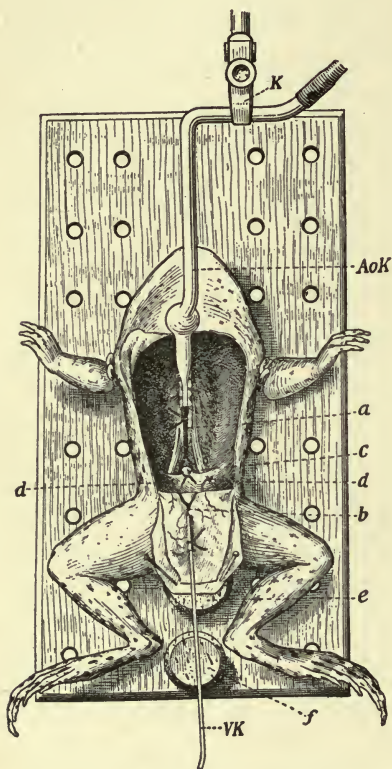


Fig. 25.—Frog preparation for vessel perfusion (Fuehner): (a) Aorta; (b) abdominal vein; (c) ligated rectum and bladder; (d) venæ renales adheventes.

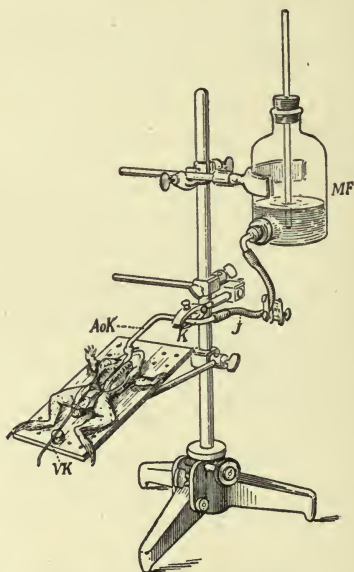


Fig. 26.—Frog perfusion (Fuehner).

After a normal tracing has been taken, $\frac{1}{2}$ or 1 c.c. of the solution to be tested is injected very slowly with a hypodermic syringe into the connecting tube. The injection should raise the fluid in the glass tube of the Mariotte bottle by about 1 cm., and should occupy about fifteen seconds. An injection of Ringer's fluid solution may first be made as a blank test to discount the mechanical effects of injection. (Tatum, 1912, Jour. Pharmacol., 4, 151, describes an arrangement for eliminating the disturbance.)

The sensitiveness of the vessels increases for several hours. The occurrence of edema is not detrimental.

TECHNICAL REFERENCES

Trendelenburg, Deut. Arch. klin. Med., 103; Arch. exp. Path. Pharm., 63, 165; *ibid.*, 1915, 79, 154; Fuehner, Nachweiss, p. 140; Tatum, 1912, Jour. Pharm. Exp. Ther., 4, 151.

The following solutions may be tried (dissolved in Ringer's fluid): Sod. Nitrite, 1 : 1000; then Epinephrin, 1 : 5,000,000; then Digitalis, 1 : 100.

(Optional) **Synergism of Epinephrin and Serum.**—Compare the following on the Trendelenburg preparation:

- (a) Ringer's solution.
- (b) Ditto, injecting 1 c.c. of Serum, 1 : 4.
- (c) Ringer's solution.
- (d) Ditto, with addition of Epinephrin, 1 : 100,000,000.
- (e) Ditto, ditto, injecting 1 c.c. of Serum, 1 : 4.
- (f) Ringer's solution.
- (g) Ditto, injecting 1 c.c. of Epinephrin, 1 : 10,000,000.
- (h) Ringer's solution.
- (i) Ditto, with addition of Serum, 1 : 150.
- (k) Ditto, ditto, injecting 1 c.c. of Epinephrin, 1 : 10,000,000. (Moog, 1914, Arch. exp. Path. Pharm., 77, 346.)

QUESTIONS

- (a) Describe the effects of these drugs.
- (b) On what structures are the actions exerted?

EXERCISE IV, B.—(OPTIONAL) PERFUSION OF ISOLATED RABBIT'S EAR

(Bissemski) Rischbieter, 1913, Zs. ges. exp. Med., 1, 355; Swetschnikow, 1914, Arch. ges. Physiol., 157, 471.

EXERCISE V.—(OPTIONAL) MICROSCOPIC OBSERVATION OF VESSELS

Experiment 1. Digitalis on Vessels of Frog's Foot.—Curarize a frog. Pin on board to observe circulation in foot (Oc. III, obj. III). Make an exact drawing of a small vessel. Inject into lymph-sac 0.5 c.c. of tincture (10 per cent.) of digitalis and observe the same vessel from time to time and note changes in its diameter. A marked vasoconstriction (about 25 per cent.) is usually observed. Ergot, 0.5 c.c. of fluidextract, may also be used.

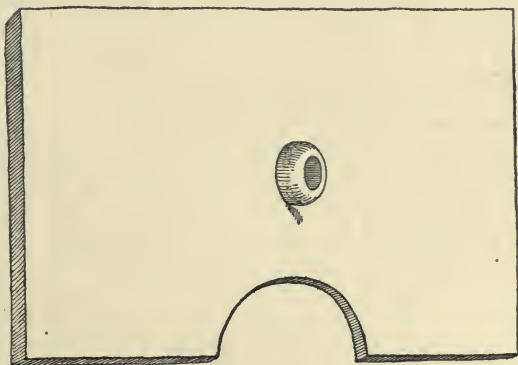


Fig. 27.—Circulation-board, for studying the circulation in the frog's omentum; $\frac{1}{2}$ actual size.

More exact results can be obtained by using a camera lucida or an eye-piece micrometer.

For observing the circulation of the frog's foot a triangular slit is cut from one end of the board, and the web of the foot is stretched over this slit. This is laid on the stage of the microscope, the other end of the board being conveniently supported by a tumbler.

Instead of using curare, the frog may be anesthetized by urethane (Oehrwall, 1911, Skand. Arch. Physiol., 25, 1).

Further descriptions of the method are found in Tigerstedt, 2,4, 312; Heinz, 2, 144; Kobert Intox., 1, 195; Cohnheim, Virch. Arch., 40.

Experiment 2. Mesenteric Vessels.—For observing the circulation in the omentum the cork-board shown in Fig. 27 is employed. A semicircle is cut out at one side to adapt it to the stand of the microscope. A hole of about 18 mm. is made near the center with a cork-borer. Into this a perforated cork (1 cm. bore) is pushed tightly. The bottom of the cork is cut off flush with the board. The top projects 1 cm. above the board. The edges of the cork are rounded with a file.

To observe the circulation, the brain of the frog is pithed. The abdomen is opened and the sciatic nerves divided within the abdomen. The frog is then pinned on the board on the side away from the microscope, so that the abdomen touches the cork. A small pledget of cotton, moistened with normal saline solution, is inserted between the frog and the cork. A coil of intestine is drawn out carefully and pinned over the cork, so that the mesentery comes to lie over the opening. Twisting of the vessels must be avoided. A triangular piece of filter-paper is laid with its base on the opened abdomen and its apex on the mesentery. This is moistened with normal saline solution.

Epinephrin, 0.01 per cent., may be tried.

The experiment may be modified to show the action of astringents by first inducing an inflammation and then applying 1 per cent. alum.

EXERCISE VI.—(OPTIONAL) BLOOD-PRESSURE OF FROGS

See Jacoby and Roemer, 1911, Arch. Exp. Path. Pharm., 66, 270; Burket, 1913, Kansas Univ. Sci. Bul., 17, 219; Kuno, 1914, Arch. ges. Physiol., 158, 1; Schulz, 1906, Arch. ges. Physiol., 115, 386; Toads, Tigerstedt, 2,4, 211.

EXERCISE VII.—(ALL GROUPS) PERFUSION OF MAMMALIAN KIDNEYS AND OTHER ORGANS

(REPORTER II, C)

Experiment 1. (Group I) Mechanical Changes in Circulation.—(See Technic, page 167.) Perfuse dog kidney with 2 per cent. NaCl, as described above, observe vein and ureter flow (drops or cubic centimeters per minute) and oncometer.

(a) *Effect of Arterial Pressure.*—Start with the reservoir at 140 cm. above the kidney. Make observations after fluid has run for about ten minutes. Lower the reservoir to 100 cm., and repeat the observations after ten minutes; also with 60 and 20 cm.

The vein flow, ureter flow, and oncometer (also the maximal vein and ureter pressure) vary in the same direction as the arterial pressure.

(By modifying the arrangement so that the pressure can be interrupted rhythmically, it can also be shown that the vein and ureter flow are much better with interrupted pressure than with constant pressure of the same mean height.)

(b) *Effect of Vein Pressure.*—Replace the reservoir at 140 cm. Remove the outflow-tip from the vein cannula and connect this with a rubber tube 1 m. long. Replace the outflow-tip in this tube and support it at the level of the kidney. Let it fill with the fluid, and in ten minutes measure the vein and ureter flow and the oncometer. Raise the vein outflow to 30 cm. above the kidney and in ten minutes repeat the observation; also at 60 and 90 cm. Increase of vein pressure increases the oncometer, but diminishes the vein and ureter flow. The diminution is gradual up to 60 or 80 cm., when there is a sharp drop.

(c) *Effect of Ureter Pressure.*—Remove the tube from the vein and connect it with the ureter cannula. Repeat the observations as in (b). The effects are similar, but the ureter pressure has a comparatively small effect on the vein flow and oncometer.

(d) *Occlusion of the Vein.*—Disconnect the tube. Count the ureter flow

and observe the oncometer. Pinch the vein tube to complete occlusion. The oncometer increases. There is a short spurt of ureter fluid, and then almost (but not quite) complete anuria (compression of the injury tubules in the boundary layer).

(e) *Injection by Renal Vein*.—Release the vein and after ten minutes count the vein and ureter flow and observe the oncometer. Change the injection tube from the artery to the renal vein: almost no fluid will run from the artery or ureter, the oncometer increasing greatly. (A valvular mechanism exists in the kidney, probably by the pressure of the distended veins on the arterial capillaries in the glomeruli.)

Questions.—(a) Describe the effects of arterial pressure.

(b) At what level does the filtration of “urine” stop?

(c) Do these facts agree with what is observed in intact animals?

(d) Describe the effects of vein-pressure.

(e) Do they agree with those in intact animals?

(f) Why does increased pressure in the renal vein diminish the filtration of “urine”?

(g) Describe the effects of ureter pressure.

(h) Is temporary anuria on compression of renal vein a valid argument against the physical filtration of urine?

(i) Can the circulation in the kidneys be reversed? Why?

Experiment 2. (Group II) Salt Actions on Kidney.—Use two bulbs connected with T-piece, one filled with 1 per cent. NaCl, the other with water.

(a) Perfuse the kidney with 1 per cent. NaCl solution, and observe the vein and ureter flow (drops per minute) and the oncometer after ten minutes.

(b) *Hypo-isotonic Solutions*.—Replace the salt solution by water. The vein and ureter flow and the volume are diminished. This is due to the swelling of the renal cells obstructing the access of the fluid to the kidney.

(c) *Hyperisotonic Solutions*.—Replace by 5 per cent. NaCl: the flow increases much above the original, the volume to about the original (lessened resistance by shrinkage of cells.)

Return to 1 per cent. NaCl solution. After fifteen minutes replace this by:

(d) *Calcium Chlorid* (1.6 per cent. of anhydrous, isotonic with 1 per cent. NaCl).—The flow and oncometer are diminished. This is a specific (ion) effect of the calcium.

(e) *Citrate*.—Replace by isotonic sodium citrate (2.75 per cent. of anhydrous): the flow and oncometer are increased. The citrate acts as a hyperisotonic solution, since it does not penetrate the cells as readily as NaCl (consult Exercise 23, No. 3).

(f) *Occlusion of Vein*.—Pinch the tube of the vein-cannula to complete occlusions. (See Experiment 1, e.)

Questions.—(a) Describe and explain the effects of hypotonic solutions.

(b) Similar effects are produced in intact animals by the intravenous sign of water; whereas the oral ingestion is diuretic. Explain the difference.

(c) Describe and explain the effects of hypertonic solutions.

(d) Does this explain that hyperglycemic animals are nearly always polyuric?

(e) Describe the effects of calcium.

(f) How can this effect be removed? Why?

Experiment 3. (Group III) Vascular Drugs on Perfused Kidney.—Use two bulbs connected with T-piece, one filled with 1 per cent. NaCl, the other with the drug dissolved in 1 per cent. NaCl.

(a) Perfuse with 1 per cent. NaCl. After fifteen minutes observe the vein and ureter flow (drops per minute) and the oncometer.

(b) *Epinephrin*.—Change to 1 : 50,000 (1 c.c. of 1 : 1000 to 50 c.c. of 1 per cent NaCl): the flow and volume are diminished (constriction of arterioles).

(c) *Hydrocyanic Acid*.—Change to 1 : 2500 HCN (2 c.c. of 2 per cent. to 100 c.c. of 1 per cent. NaCl): increase of vein, ureter, and oncometer (dilation of arterioles). This effect of hydrocyanic acid seems to be confined to the kidney.

(d) *Digitalis*.—Change to 1 : 1000 Digitalis (1 c.c. of 10 per cent. to 100 c.c. of 1 per cent. NaCl): vasoconstriction.

(e) *Chloral*.—Change to 1 : 1000 Chloral (1 c.c. of 10 per cent. to 100 c.c. of 1 per cent. NaCl): vasodilation.

(f) *Barium*.—Change to 1 : 2000 Barium Chlorid (5 c.c. of 1 per cent. to 100 c.c. of 1 per cent. NaCl): vasoconstriction.

Questions.—(a) Which of the drugs produce vasoconstriction?

(b) Which produce vasodilation?

Experiment 4. (Group IV) Blood and Drugs on Excised Kidney.—Use two bulbs connected with T-piece, one filled with 1 per cent. NaCl, the other with the blood, etc.

(a) Perfuse with 1 per cent. NaCl. After fifteen minutes observe the vein and ureter flow (drops per minute) and oncometer.

(b) *Blood*.—Dilute with about three volumes of 1 per cent. NaCl: the vein flow is promptly increased, while the ureter flow and oncometer are greatly diminished. Note the darkening of the venous blood. The flow is again somewhat slowed after a time.

Two factors are concerned in these effects: The great viscosity of the blood, which would slow the flow and diminish the volume. In dead kidneys the vein flow is practically arrested. In living kidneys, however, the blood stimulates a vasodilator mechanism, probably in the efferent arterioles, which causes the vein flow to continue, and generally increases it above normal.

(c) *Saline Diuretics*.—After about fifteen minutes repeat the observations. (The vein flow will be somewhat slowed on account of the increasing viscosity.) Add about 30 per cent. of 1 per cent. NaCl to the perfusing blood: the flow and volume are increased (lessened viscosity, and consequently lesser resistance). Use this blood dilution in all subsequent experiments.

(d) *Caffein*.—Substitute diluted blood with 1 : 5000 Caffein (1 c.c. of 1 per cent. to 50 c.c. of diluted blood): somewhat increased flow and volume. (Not always successful.)

(e) *Hydrocyanic Acid*.—Substitute blood with 1 : 2500 HCN (2 c.c. of 2 per cent. to 100 c.c. of diluted blood): further vasodilation.

Note that the venous blood is not darkened, but that it is readily reduced by ammonium sulphid. (Cyanids prevent the reduction of blood by paralyzing the oxygen-consuming metabolism of the cells.)

(f) *Digitalis*.—Substitute blood with 1 : 1000 Digitalis (1 c.c. of 10 per cent. to 100 c.c. of diluted blood): strong vasoconstriction. (More dilute solutions cause some dilation.)

Questions.—(a) What effect has blood on the renal circulation?

(b) Describe the effects of adding saline solution.

(c) How do these compare with those of hydremia in intact animals?

(d) How does this explain increased diuresis when effusions are being absorbed?

- (e) Describe the typical effects of caffein.
- (f) Could the diuretic action of caffein be due to its action on the kidney vessels?
- (g) Describe the effects of hydrocyanic acid.
- (h) What changes does it cause in the color of the blood?
- (i) How are these explained?
- (k) What effect would such an action have on an animal?
- (l) Describe the effects of digitalis.

Experiment 5. (Group V) Circulation Through Excised Spleen or Intestine.—In this exercise the drugs are injected slowly into the circulation by means of a hypodermic syringe. The experiment may be modified by adding the drugs directly to the perfusing fluid, and by using cold or warm defibrinated blood.

Perfuse with 1 per cent. NaCl and in ten minutes observe the rate of flow (drops per minute) and oncometer:

Suprarenal.—Inject 5 c.c. of 1 : 10,000 epinephrin: vasoconstriction.

Nitrites.—Inject 5 c.c. of 1 : 100 sodium nitrite: vasodilation.

Digitalis.—Inject 5 c.c. of 1 : 100 digitalis: vasoconstriction.

Chloral.—Inject 5 c.c. of 1 : 100 chloral: vasodilation.

Barium.—Inject 5 c.c. of 1 : 1000 barium chlorid: vasoconstriction.

Instead of injecting the stronger solutions, weaker concentrations may be perfused as in Experiment 3:

Epinephrin, 1 : 50,000 = 2 c.c. of 0.1 per cent. to 100 c.c. of 1 per cent. NaCl.

Sodium Nitrite, 1 : 2000 = $\frac{1}{2}$ c.c. of 10 per cent. to 100 c.c. of 1 per cent. NaCl.

Digitalis, 1 : 1000 = 1 c.c. of 10 per cent. to 100 c.c. of 1 per cent. NaCl.

Chloral, 1 : 1000 = 1 c.c. of 10 per cent. to 100 c.c. of 1 per cent. NaCl.

Barium Chlorid, 1 : 20,000 = $\frac{1}{2}$ c.c. of 1 per cent. to 100 c.c. of 1 per cent. NaCl.

EXERCISE VIII.—(ALL GROUPS) AMYL NITRITE ON CIRCULATION, MAN

(REPORTER III, C)

The circulatory reactions of man may be studied by ordinary clinical methods, but normal men under the disturbing conditions of the laboratory class are not good subjects for the usually delicate changes.

Amyl Nitrite, however, gives results which are sufficiently positive. It is administered by inhaling 3 drops from a handkerchief.

Experiment 1. (Group I) General Symptoms.—Note the beginning and duration of the effects. Observe the throbbing of the head; the extent of the blush; the changes in pulse-rate and respiration.

Experiment 2. (Groups II and III) Blood-pressure.—Observe with one of the clinical instruments.

The pressure in the cuff is raised until the pulse disappears, and then slowly released until the critical points are reached. In the Korotkow auscultatory method the stethoscope is applied peripheral to the cuff, when the following sounds appear successively as the pressure is released:

- (1) Somewhat like the first cardiac sound. This indicates the *systolic pressure*.
- (2) The above sound, with a hissing murmur.
- (3) The murmur disappears, leaving only the sound.
- (4) The sound suddenly becomes muffled, and (5) disappears. (4) and (5) indicate the *diastolic pressure*.

Experiment 3. (Groups IV and V) Plethysmograph.—Take plethysmographic tracing.

Experiment 4. (Optional) Sphygmograph.—Take tracing.

QUESTION

Describe the effects of amyl nitrite.

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Human Blood and Plasma Volume.—"Vital red" method; Keith, Rowntree, and Geraghty, *Arch. Int. Med.*, 16, 547.

Plethysmographs.—The attachment of the cuff to the arm may be sealed with petrolatum; especially in hairy animals. The plethysmograph may be set on sand, to avoid vibration. Air transmission gives the best results.

Recording Devices for Plethysmographs.—Besides the usual piston and bellow recorders, special devices are described by Schlager, 1906, *Cbl. Physiol.*, 20, 257; Strassburger, (Spirometer), *Arch. ges. Physiol.*, 139, 33; Dixon, 1907 (frog intestine), *Proc. Physiol. Soc.*, Feb. 23.

EXERCISE IX.—(OPTIONAL) ARTIFICIAL CIRCULATION SCHEMA

The effect of changes in the heart and blood-vessels on the blood-pressure and blood-flow can be demonstrated in an instructive manner by the circulation model depicted in Fig. 28.

Make the following observations and record them in tabular form. The time can be kept with a metronome. The pumping should be continued for a short time before observations are taken.

Students A and B, reading of arterial and venous pressure; students C and D, pumping and outflow; students E and F, recording.

	Arterial pressure.		Venous pressure.		Outflow (at v.).
	Max.	Min.	Max.	Min.	(Time required for 100 c. c.)
1. (<i>Normal</i>) Pump with moderate excursions; at rate of 60 per minute. The capillaries-clamp is partly closed.					
2. (<i>Vagus Stimulation</i>) Pump at the rate of 10 per minute, allowing complete relaxation, but incomplete contraction.		Falls.	Falls.		Falls.
3. (<i>Vagus Depression</i>) Pump at the rate of 120 per minute, but with very weak compression.		Little rise.	Little rise.		Little rise.

	Arterial pressure.		Venous pressure.		Outflow (at v.). (Time required for 100 c.c.)
	Max.	Min.	Max.	Min.	
4. (<i>Digitalis</i> Action on Cardiac Muscle) Pump at the rate of 30 per minute, causing complete contraction, but incomplete relaxation....	Rises.		Rises.		Rises.
5. <i>Simultaneous Stimulation of Vagus and Cardiac Muscle</i> (<i>Digitalis</i>).—Pump at the rate of 30, with complete contraction and relaxation.....	Rises.		Rises.		Rises.
6. <i>Vasoconstriction</i> .—Repeat 1, then tighten the capillaries-clamp.....	Rises.		Falls.		Falls.
7. <i>Vasodilation</i> .—Open the capillaries-clamp....	Falls.		Rises.		Rises.
8. <i>Complete Digitalic Action</i> .—Combine 5 and 6.	Rises more than 5 or 6.		Rises less than 5, more than 6.		Rises less than 5, more than 6.

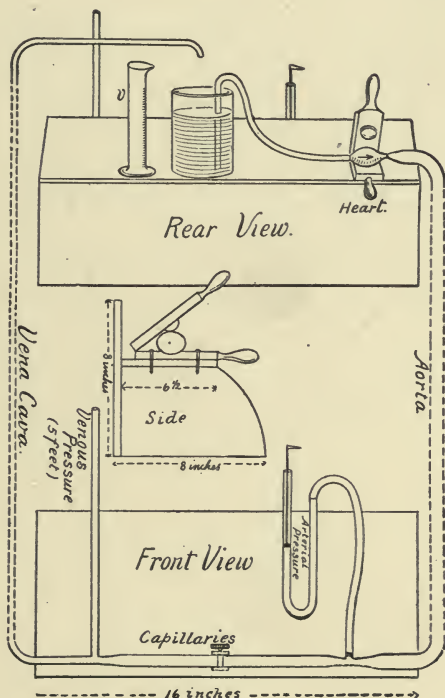


Fig. 28.—Artificial circulation model. The heart is represented by a rubber syringe bulb with valves in the direction of the arrow. This is compressed by a lemon-squeezer. The vessels are formed by rubber tubing, that for the aorta being especially elastic. The arterial pressure is taken on a mercury manometer; the vein pressure by an upright tube filled with water. The capillary resistance is furnished by a screw-clamp. The dimensions of the apparatus are indicated on the figure.

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Artificial Circulation Schemes, Tigerstedt, 2.4, 319.

CHAPTER XXXVI

EXCISED AND FROG HEARTS

INTRODUCTORY

The Heart Muscle.—Automaticity.—The cardiac muscle differs from other muscle by the fact that it contracts rhythmically by an inherent property, *i. e.*, even in the absence of nervous impulses.¹ This property is sometimes called the *automatic motor mechanism* of the heart. If the heart is weakened, it may be lost so that the heart may respond to stimulation by a single contraction, just like ordinary muscle. On the other hand, the rhythmic property may be imparted to ordinary muscle; for instance, by immersing it in certain solutions of NaCl. The rhythmic property, therefore, does not constitute a fundamental distinction between cardiac muscle and the other varieties of muscle, although under normal conditions it is a very important difference. The other properties of cardiac muscle are still more closely related to those of other muscle: its excitability, strength of contraction, tonus, etc., may be similarly affected by fatigue or by drugs; in these respects the myocardium stands intermediate between the skeletal and the smooth muscle. Normally the rhythmic contractions arise in the base of the heart—in the auricles, or in the frog in the sinus venosus; and spread gradually to the apex. Consequently the *contractions* are *regular*, progressing in a definite order, and all parts of the heart beat at the same rate, and the two sides of the heart contract at the same time. The explanation of these facts is that the muscle-fibers at the base of the heart are more excitable, so that they respond first to the (inherent) rhythmic stimulus; the successive areas of the ventricles contract then as the result of the stimulus started by the contraction of the auricles.

Irregularities.—If the excitability of the ventricle is increased as the result of the action of drugs (such as digitalis, caffeine, or aconite), the contraction may start in any part of the heart. *The normal rhythm is thereby destroyed*, the contractions cease to progress regularly, and the rate of each chamber of the heart may differ from the others. If the contractions arriving from the ventricles coincide with those transmitted from the auricle, the contractions are strong; if they interfere, the contractions may be weak or absent. In this way *groups* of strong contractions may alternate with periods of weak contractions. A *decrease of excitability* finds its first expression in the more sluggish ventricles. As a consequence, a summation of two or more auricular contractions may be necessary to induce a contraction of the ventricle, and the rate of the latter may be a fraction of the auricular rate. This is seen with cardiac depressants, especially in the frog's heart.

Another form of irregularity, observed particularly in frogs as the result of digitalis or aconite, consists in *peristaltic contractions*, in which the slowly traveling contraction wave is sharply marked off. This may be due to a quicker contraction, with delayed relaxation.

Delirium Cordis.—If the cardiac muscle of mammals is overstimulated the contractions become very irregular. The individual groups of muscle-fibers contract independently (hence *fibrillary contractions*), while the heart as a whole does not perform any efficient contractions. This condition, also called *delirium cordis*, appears to be an overquickening; it takes the place of the tetanus of the striped muscle, the mammalian heart being ordinarily unable to enter into tetanus on account of its rhythmic property.² The ventricles enter into delirium more readily than the auricles because the latter are capable of a more rapid rhythmic beat, so that overstimulation is not reached so easily. The frog's heart also does not readily go into delirium because it is too sluggish for overstimulation; but when its excitability is raised—as by heat—delirium can be produced.

Since the delirious heart does not keep up an efficient circulation the mammalian heart (which is nourished by the coronary circulation) is starved and succumbs rapidly to fatigue. Delirium ordinarily produces paralysis of the heart unless the coronary circulation is sustained artificially. The rabbit's heart may recover spontaneously; the dog's heart does not.

Coronary Circulation.—The state of the *coronary circulation* is very important for the mammalian heart, as its great activity demands a liberal nutrition. The effect is mainly upon the strength of the contractions, the rate being but little altered. Consequently, all agencies which depress the heart directly also depress it indirectly by lessening its food supply, and vice versa. An excessive tonus of the heart by lessening the excursions also

¹ In the heart of Limulus (King Crab) and perhaps in some other invertebrates the rhythmic impulses are generated and conducted by nerves (Carlson, 1904).

² Tetanus of the mammalian heart can be produced only by the simultaneous stimulation of the vagus and cardiac muscle.

starves the heart, so that a strong stimulation of the cardiac muscle may rapidly paralyze it by interfering with the coronary circulation; and systolic standstill is consequently impossible in the intact mammalian heart since the starved muscle cannot sustain the systole.¹ A strong contraction of the myocardium also causes a mechanical compression of the coronary vessels, thereby lessening the blood-flow through them. On the other hand, extreme dilation of the heart also lessens the coronary circulation; so that an overdistended heart may often be improved by withdrawing some blood. Since the coronary vessels possess vasoconstrictor and vasodilator nerves, they may be affected by drugs acting centrally or peripherally on the vasomotor mechanism.² The coronary circulation may also be modified *indirectly* through changes in the general arterial pressure. Vasoconstrictors will therefore stimulate the heart,³ and vasodilators will depress it. In the excised heart these agents may have the opposite effects, since they act then on the coronary arteries alone; but in the intact animal the effects on the circulation at large will overcome the effect on the muscle of the coronary arteries.

Changes in blood-pressure have also a mechanical effect on the heart: the cardiac muscle, like other muscles, contracts better against a certain resistance than against no resistance. This resistance is furnished by the aortic pressure. The normal blood-pressure seems to furnish the optimum resistance to the normal heart, so that it would be a mistake to consider that a fall of pressure, by lessening its work, would increase the force of a normal heart. With a *weakened* heart, however, the optimum resistance falls, so that a diminished pressure is really beneficial to an exhausted heart.

The *amplitude of the contractions* is controlled not only by the force of the heart, but also by its tonus, by its rate, and by the blood-pressure. A tonus which is greatly increased or diminished will prevent the muscle from relaxing or from contracting to the usual extent. An increased rate does not allow time for complete contraction and relaxation, and so renders the beats more shallow, while a slow rate tends to increase the excursions. A high blood-pressure prevents the complete emptying of the heart, and thereby renders the beats more shallow and slows the rate. (In intact animals this slowing is very marked, being due to a reflex stimulation of the vagus mechanism.)

The *volume of blood thrown out at each beat* varies with the amplitude of the excursions. The *output in a given time* is the product of the rate and the volume of each beat. The *work done by the heart* is the product of the output and the resistance (blood-pressure) against which it acts.

Effect of the Rate of the Heart on the Output.—The output of the heart is greatly diminished by slowing its ordinary rate; the increased volume of each beat being insufficient to counterbalance the lessened number of contractions. A quickening of the heart above the normal, on the other hand, causes but little increase in the output, since the lessening of the volume of each beat nearly offsets the increased rate. This, as well as the effect of the vascular system, etc., may be demonstrated on an artificial circulation apparatus.

The Innervation of the Heart.—Although the cardiac muscle is able to perform regular rhythmic contractions in the absence of nerves, it is normally under nervous control. Besides the sensory (depressor) nerve there are two motor nerves, the vagus and the accelerator branch of the sympathetic. The origin (center) of both of these nerves is in the medulla. Both nerves run in the same sheath in the frog, but are separated in mammals. Both nerves are connected with ganglia. Those of the vagus are contained in the heart itself (in the frog these vagus ganglia are situated especially at the juncture of the sinus venosus with the auricle). Those of the accelerator are extracardiac, and in mammals lie probably in the inferior cervical and in the stellate ganglia, around the subclavian artery. The endings in the cardiac muscle are “free endings,” similar to those of unstriated muscle. The heart contains no structures corresponding to the end-plates of striated muscle.

The effect of *electric stimulation* of these nerves appears only after a slight latent period, and disappears after a time, even if the stimulation is continued. The latent period and the action are longer for the vagus.

Moderate vagus stimulation causes a slowing of the rate, the diastole being especially prolonged. The irritability and the contractile power are increased in mammals; the amplitude of the excursions is larger; the tonus is diminished; the blood-pressure and output fall. *Strong stimulation* causes diastolic standstill.

¹ The mammalian heart is, however, capable of systolic standstill if the coronary circulation is maintained by artificial means, as in Langendorff's method.

² In working with artificially perfused hearts it is important to remember that the coronary vessels are also affected by the *temperature of the blood*, being dilated by heat and constricted by cold. The temperature has an even greater effect on the cardiac muscle itself, heat quickening the rate, while cold slows the contractions.

³ Rhythmical beats may be produced in the excised mammalian heart by simply raising the intracoronary pressure by indifferent gases (hydrogen, Magnus, 1902) or oil (Sollmann, 1906).

Accelerator stimulation (the anterior ramus of the annulus of Vieussens) quickens the rate, shortening all the phases except the auricular systole and the ventricular diastole. The excitability, tonus, and strength of the heart are increased, but the pulse is more shallow in mammals (in the frog the excursions are also increased). The blood-pressure and the output rise somewhat, but not commensurate with the increased rate.

Tonic Impulses.—The vagi are tonically active in some animals (notably in man and in dogs), so that division of these nerves causes a quickening of the heart. In other animals (rabbits) there is normally no tonic action, so that division produces little effect on the heart rate. The accelerators are also tonically active, but division of these nerves produces less effect than section of the vagi. The ganglia and endings also have some tonic action, for a further quickening may be obtained, after section of the vagi, by paralyzing the vagal endings.

The vagi or accelerators may be stimulated or depressed directly at their origin, or in any part of their course, by drugs or by other means. They can also be affected *indirectly*, especially the vagus. A fall in blood-pressure, most forms of reflex irritation, muscular exercise, swallowing, etc., quicken the heart by inhibiting the vagus center. Stimulation of the trigeminal endings, on the other hand, excites the vagus center and slows, or even stops, the heart. A rise of blood-pressure also stimulates the vagus and causes slowing.

Methods of Studying the Actions of the Heart.—It follows from the preceding that the action of the heart depends on a considerable number of interrelated factors. These, acting together, produce the phenomena which may be studied on normal animals by the pulse and apex-beat; and on operated animals by direct observation and tracings of the exposed heart. The intracardiac and the general blood-pressure and the output of the heart, etc., are also determined in large part by the cardiac activity; but since they also depend on the state of the vasomotor system, they must be supplemented by more direct methods. Indeed, all the observations on intact animals give only the sum of the factors which may be involved. It is evident that no understanding of the action of a drug is possible until the share of each factor is known. This must be determined by isolating it as completely as possible from the other factors.

Suitability of Different Animals.—The hearts of frogs and turtles are convenient for studying the effects of drugs, since they continue beating normally for a considerable time after they are exposed or excised. Many phenomena can be observed very well by direct inspection or by perfusion, others may be recorded by levers, etc.

The *cardiac nerves* of frogs are also situated conveniently. It must not be forgotten, however, that the *physiology of the heart of cold-blooded animals differs considerably from that of the warm-blooded*; and caution must be used in applying the results obtained with the one to the other. The main uses of the frog's heart are, therefore, restricted to preliminary studies, to the investigation of special problems, and to the convenient demonstration of actions which have been already controlled on warm-blooded animals. Among the latter the functions of the myocardium are identical, as far as we know. The absence of tonic vagus impulses in rabbits must be borne in mind.

Drugs may act on the heart in three ways: (1) Directly on the cardiac muscle; (2) directly on the cardiac nerves, and (3) indirectly, on either the muscles or nerves—through reflexes, altered resistance, altered nutrition, altered coronary circulation, etc.

Methods of Studying the Direct Effects on the Cardiac Muscle.—These demand that the resistance to the work of the heart be kept constant—an object which can only be accomplished by separating the heart from the general vascular system. The pulmonary circulation may generally be kept intact, as it is not much affected by drugs. The methods of isolating the heart may, however, be conveniently divided into those which retain the pulmonary circulation and those which do not. The nervous mechanism should also be excluded. If it is desired to retain the intracardiac nervous apparatus, it suffices to cut the trunks of the vagi and accelerators, or to shut off the blood from the medulla. The intracardiac vagus mechanism can also be paralyzed by atropin. This leaves only the accelerator endings.

The frog's heart will continue to beat for some time after it has been excised from the body; but the mammalian heart requires that the coronary circulation be maintained. This may be done by the heart itself, or by injecting the perfusion fluid under pressure.

Perfusion Liquids.—In perfusing the excised heart a fluid must be employed which does not produce any salt or ion action, which contains oxygen and nutriment, and which is at body temperature. The best is oxygenated defibrinated blood from the same species of animal, diluted with 5 volumes of Locke's solution. Other fluids may be substituted, but these must be charged with oxygen when used with the mammalian heart. Serum may be employed. An excellent substitute is *Locke's Fluid*. By the use of Langendorff's or Porter's method the heart can be kept beating, or revived, many hours after death.

Similar solutions may be used for the perfusion of frogs' hearts, except that they should contain less salt (0.6 to 0.75 per cent. NaCl). Used alone, this saline solution gradually poisons the heart after the manner of digitalis. The toxicity is less if 2 per cent. of gum arabic is added, or small quantities of some other salts. *Ringer's Solution* (see Index) has been found very good. Rabbit's or beef's blood, defibrinated and diluted with $2\frac{1}{2}$ parts of 0.6 per cent. NaCl, is also used.

Analysis of the Effects on the Heart.—Actions on the nervous mechanism can be studied with the heart *in situ* by dividing or stimulating the vagi and accelerators at different levels. Cyon has also devised a method of studying the effects of drugs upon the cerebral cardiac centers by separating these from the general circulation and artificially circulating through them defibrinated blood containing the poisons to be studied; in this way they do not reach the heart at all.

An effect upon the nerves is manifested particularly by changes in the rate of the heart; but as the rate may also be modified through the muscle, or indirectly, a more detailed analysis becomes necessary; this will repay closer study, as it illustrates the methods of pharmacologic research.

Investigation of Changes in the Rate of the Heart.—**Quickening** may be due to a direct or reflex inhibition of the vagus, or to stimulation of the accelerator nerves or of the cardiac muscle.

(1) If the quickening does not occur after section of the vagi, it must have been due to *central paralysis of the vagi*. If the center does not respond to reflex stimulation (such as the inhalation of ammonia with rabbits), the center itself is paralyzed. If it does respond, the inhibition of the vagus must be *reflex*, which can be further demonstrated by division of the corresponding path.

(2) If the quickening occurs after section of the vagi, the drug is tried on animals in which the vagus endings have been completely paralyzed by atropin. If it produces no effect, the drug must paralyze either the ganglia or endings. It is tried on animals in which the ganglia have been paralyzed by nicotin; or on the ganglion-free apex of the frog's heart. If it produces no quickening, it must have *paralyzed the vagus ganglia*; if quickening occurs, it must *paralyze the endings*. In the former case, stimulation of the sinus, in the frog, stops the heart; if the endings are paralyzed stimulation of the sinus has no effect.

(3) If the quickening occurs even after atropin, there must be a stimulation of either the accelerator mechanism or of the cardiac muscle. If the effect occurs on the *excised* atropinized heart, it must stimulate either the *muscle or the accelerator endings*. It is very difficult to distinguish between these; the study of the relative duration and strength of the phases of the cardiac cycle furnishes some indication. The cardiac muscle, quite free from nerve-endings, can also be studied in the embryonal chick. It appears, from these methods, that the stimulation is always of the muscle rather than of the endings, so that we shall designate a quickening obtained after atropin as a *stimulation of the cardiac muscle*.

(4) If the drug acts after atropin, but has no effect on the excised heart, it must *stimulate the accelerator center*. This can be further shown by its producing no effect on the intact animal if the spinal cord is divided above the first dorsal vertebra, or if both stellate ganglia are excised.

Slowing may be due to direct or reflex stimulation of the vagi; to paralysis of the accelerators; to paralysis of the muscle, direct or through impaired nutrition; or to systolic stimulation of the muscle.

1. If the slowing does not occur after section of the vagi, it must be due to a *stimulation of the vagus center*, especially if electric stimulation of the vagus trunk continues effective.

2. If it occurs after section of the vagi, but not after nicotin, it must be due to *stimulation of the vagus ganglia*.

3. If it occurs after section of the vagi and after nicotin, but not after atropin, it must be due to *stimulation of the vagus endings*. Electric stimulation of the vagus trunk is ineffective in 2 and 3.

4. If it occurs after atropin, but not after division of the accelerators, it must be due to a *depression of the accelerator*. If electric stimulation of the accelerator nerve is effective, the depression must be *central*; if not, it is *peripheral*.

5. If it occurs after atropin and after division of the accelerators, it must be due to a direct action on the cardiac muscle, or to *insufficient nutrition*. The latter may be excluded by artificial circulation. If the slowing persists, it is due to *paralysis*, or to *increased tonus*, of the cardiac muscle; the strength of the contractions will indicate which is the true explanation.

Cardiac standstill may be due to stimulation of the vagus, to paralysis of the cardiac muscle, and (in frogs) to excessive systole.

1. If the standstill disappears on section of the vagi, it is due to *stimulation of the vagus center*.

2. If it persists, but disappears after atropin, it is due to *peripheral stimulation of the vagus*. The ganglia and endings can be distinguished as in "Slowing," 2, 3. The frog's heart is strongly diastolic if the stoppage is due to stimulation of any part of the vagi.

3. If atropin does not relieve the standstill, it is caused by a *direct effect on the muscle*. In mammals, this is always paralytic. In frogs it may be due to *paralysis*, when the heart is of medium size, and cannot contract if it is forcibly distended; or to *excessive systole* (Digitalis group) when the heart is very small, and contracts if distended.

4. The paralysis may only involve the *rhythmic power*, so that the heart responds to stimulation (*i. e.*, a pin-prick) by a single contraction; or it may be complete.

EXERCISE I.—(DEMONSTRATION) EXCISED MAMMALIAN HEART (LANGENDORFF METHOD)

(REPORTER II, A)

The method consists essentially in perfusing the coronary vessels of the excised heart with a warm oxygenated saline solution. Various arrangements may be used, the following being one of the simplest, but not sufficient for exact work:

Perfusion Apparatus (See Fig. 29).—A large water-bath, *w.b.*, heated by a Bunsen or alcohol burner, is arranged on a shelf 150 cm. above the table. In this is set a 2-gallon

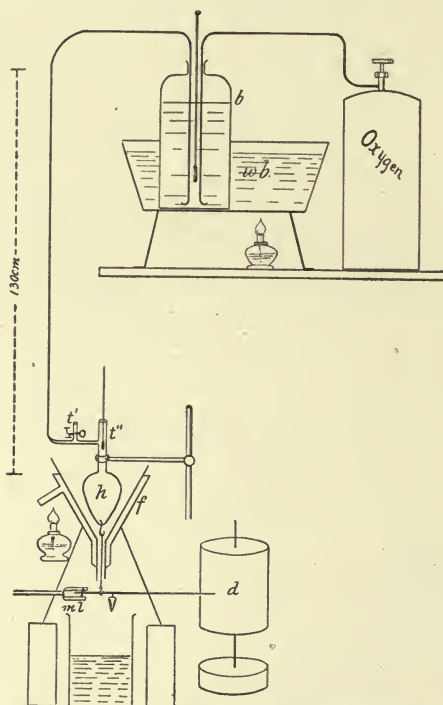


Fig. 29.—Apparatus for Langendorff heart (see text).

bottle containing 7 liters of fresh Locke's fluid. Into this bottle dips a siphon, a narrow orifice tube connected with the oxygen-tank, and a thermometer. The siphon tube is prolonged to the table. A T piece, t'' , is inserted near the lower end, the free limb being

¹ This serves for the removal of air or of cooled blood, if the flow has been arrested.

closed by a Mohr clamp. The tube terminates in another T, *l''*, which bears the bulb of a thermometer. This T is joined to the aortic cannula, and supported by a clamp and stand, over the hot-water funnel *f*. This is kept warm by a Bunsen or alcohol flame. A pin is hooked to the apex of the heart, *h*, and connected with a string, which passes through the stem of the funnel to a muscle lever, *m.l.*, writing on the drum *d*. The lever is weighted with a 10-gm. counterpoise. It is best to attach the string to the lever with a pin, so that the excursions can be regulated to $1\frac{1}{2}$ or 2 inches. A beaker is set beneath the funnel to catch the blood. Several drums should be smoked in advance. The whole apparatus should be ready before the heart is excised.

Preliminary Operations.—While the apparatus is being set up the dog is anesthetized, and cannulae are tied in the trachea, carotid, and femoral vein. The latter is connected with the injection buret. The dog is now bled from the carotid as long as the blood-flow is a strong stream. The carotid is clamped, and the blood is defibrinated, strained, and heated to 45° C. and poured into *b*. The heart is now exposed and artificial respiration is started. The carotid is again opened, and the dog is bled, while at the same time a liter of warm Locke's fluid is allowed to flow into the femoral vein.¹ The diluted blood is collected as long as it flows from the carotid, defibrinated, strained, mixed with the blood which was previously drawn, heated to 45° C., and poured into the reservoir.

The reservoir is now shaken so as to mix the fluids, and a slow stream of oxygen is passed through it. The siphon tube is filled with the blood.

In the meantime the heart is excised with an inch of the aorta, and with the lungs. The latter are trimmed away, the pericardium is slit open. All branches of the aorta are tied. The aortic cannula is introduced and secured by a firm ligature, taking care that it does not interfere with the play of the semilunar valves. The aorta is clamped below the cannula; this is filled with blood, connected air-free with *l'*, and supported in the clamp. The pin is hooked into the apex, connected with the lever, the clamp on the aorta is removed, and the perfusion is started. The pressure closes the semilunar valves, so that the fluid is forced through the coronary circulation, escaping through the right auricle and into the beaker. The flow should be rather free, the beaker being frequently exchanged, the unpoisoned blood being returned to the reservoir. If it is too free, some of the veins may be closed by bulldog forceps. See that the thermometer in *l''* registers 38° to 42° C.

The heart will begin to beat in a very short time, at first feebly and irregularly, but soon with strong, regular beats. The observations and tracings may be started at this time. The solutions should be injected just below *l'* with a hypodermic syringe, thrust obliquely through the rubber. The injections should be made *very slowly*, and continued until the desired effect is obtained.

(Instead of injecting the drugs with a syringe, they may be added directly to the perfusing fluid, in the proportion of about 1 : 25. A second reservoir will be necessary.)

Experiments.—1. **Strychnin.**—Obtain a normal tracing. Inject 1 : 5000 Strychnin. According to the dose (which is really inversely proportional to the rate of perfusion), one may obtain:

- (a) No effect.
- (b) Increased excursions.
- (c) Diminished excursions.

2. **Caffein.**—Inject 1 : 5000 Caffein. According to the dose, one may obtain quickening and increased excursions; or slowing with diminished excursions.

3. **Chloroform.**—Inject a saturated solution of Chloroform in normal saline: the heart is slowed and especially weakened. Proceed to 4 before it has quite stopped.

4. **Epinephrin.**—Inject 1 : 30,000: the heart revives promptly and beats powerfully.

5. **Potassium.**—Inject 1 : 100 KCl: sudden paralysis of the heart. Recovery may be spontaneous or occur by 6, which should be undertaken at once.

6. **Camphor.**—Inject a saturated solution of Camphor in normal saline solution: the heart revives or is strengthened.

¹ Salant and Hecht, 1915, Amer. Jour. Physiol., 36, 130, claim that the heart behaves much better if it is excised without previous bleeding.

7. Digitalis.—Inject 1 : 100: the heart is first quickened and strengthened. The tonus increases. Finally it goes into delirium cordis and stops in systolic position.

(Optional) Concentrations of Various Drugs for Direct Perfusion (Greene).—Aconite, 0.0002 per cent.; Alcohol, 0.4, 1, and 2 per cent.; Atropin, 0.001 per cent.; Caffein, 0.02 to 0.5 per cent.; Chloroform, 0.02 to 0.05 per cent.; Digitalis, 0.0001 and 0.0005 per cent.; Ether, 1 per cent.; Morphin, 0.5 and 1 per cent.; Nicotin, 0.001 per cent.; Physostigmin, 0.01 per cent.; Strychnin, 0.005 and 0.02 per cent.; Veratrin, 0.002 per cent.

TECHNICAL REFERENCES

Fuller descriptions are given in Langendorff, 1895, Arch. ges. Physiol., 61, 291; Stewart, 203; Pittenger, 126; Tigerstedt, 2.4, 144; Kobert, Intox., 1, 180; Greene, 73; Abderhalden, 3, 333 (metabolism, *ibid.*, 374).

The following modifications and improvements may be mentioned: Herlitzka, 1905 (Constant Pressure), Arch. ges. Physiol., 107, 564; Locke and Rosenheim, 1907 (Continued Perfusion with Small Quantities), Jour. Physiol., 36, 205; Brodie and Cullis, 1908 (Uniform Temperature and Small Dead Space), Jour. Physiol., 37, 337; Eyster and Loewenhardt, 1913, Jour. Pharmacol., 5, 57; Dresbach, 1913, Quart. Jour. Exp. Physiol., 8, 73; A. J. Gunn, 1913, Jour. Physiol., 46, Aug. 18 (good arrangement for heating the injection fluid).

Technical Notes.—Outline of Methods for Studying the Isolated Mammalian Heart.—The methods which have been employed for the study of the isolated mammalian heart are briefly as follows:

1. Methods Employing the Whole Heart and Pulmonary Circulation (Excluding the Peripheral Vessels and, to a Large Extent, the Brain—Fig. 30).—These methods differ by the manner in which the action of the heart is observed or recorded, which may be done by direct observation, by taking pressure curves from the carotid or from the ventricles, or by the myocardiogram. The methods consist essentially in establishing a connection between the large arteries and large veins, and then ligating the vessels peripherally to this connection. The vessels which are employed for this purpose and the apparatus used for establishing the connections vary in the different methods.

(A more recent "isolated lung-heart preparation" for dogs is described by Knowlton and Starling, 1912, Jour. Physiol., 44, 206; 45, 146.)

(a) *Communication established between the aorta and right auricle:*

1. *Martin's Original Method.*—In this a communication is established through a reservoir containing defibrinated blood and connected with the right auricle, while the left ventricle pumps the blood through a tube back into the reservoir. The course of this blood then is: right auricle, pulmonary circulation, left heart, standing tube, and reservoir. The oxygenation of the blood is effected by artificial respiration.

2. *The modified method of Martin and Applegarth* establishes a communication through the coronary vessels, the maintenance of pressure being aided by connection of the aorta with a reservoir containing defibrinated blood. The course of the blood is: aorta, coronary circulation, right heart, lungs, left heart, and aorta. Oxygenation is by artificial respiration.

3. *The McGrath and Kennedy method* is an amplification of the last, in that it measures the intracardiac pressure and the outflow through the pulmonary artery.

4. *Hedon and Arrous' method* differs from the preceding methods by leaving out the reservoir, simply tying the aorta and its branches and the vena cava. The course of the blood is: aorta, coronary circulation, right heart, pulmonary circulation, left heart, and aorta. Oxygenation is by artificial respiration.

The heart survives some hours. It becomes progressively slower by the using up of material and the production of waste products, but it remains regular.

5. *Cyon* connects the aorta with the vena cava. In addition, he is very careful to ligate all the vessels leading to the brain, so that he can expose this organ to poisons without their reaching the general circulation.

(b) *Communication Through the Carotid and Jugular.*—The methods differ mainly in the mechanism introduced as resistance, this being either constant or variable:

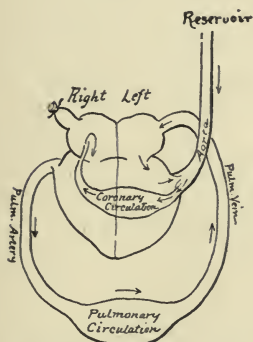
1. *Stolnikow* makes the connection through two glass vessels of known content, which are reversible, and one of which is alternately filled by blood expelled from the heart, while the other empties into the vena cava. In this way the volume of blood expelled by the heart in a given time can be measured. The other vessels are, of course, ligated. Oxygenation is by artificial respiration.

2. *Bohr and Henriquez* establish the connection by a simple tube. *Hering* does not ligate the veins, using them as a pressure regulator. *Bock* forms the connection through

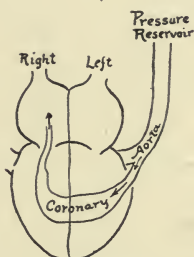
a compressible tube and screw-cock, so that a varying resistance may be introduced. He describes a rather complicated improvement of the method in Arch. exp. Path. Pharm., 1908, Suppl., 83.

In all these methods the registration is done by a manometer in the other carotid, the aorta and vena cava being ligated and artificial respiration being kept up.

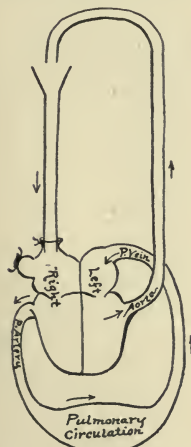
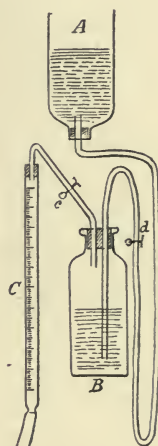
II. **Completely isolated hearts, i. e.,** without the pulmonary circulation, but with the ganglia still active. In these methods the blood must be artificially oxygenated, and is usually introduced under pressure. Otherwise the methods are similar to the preceding.



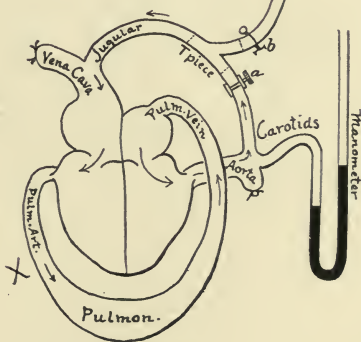
Martin and Applegarth. *Tschilowitch* connects the pulmonary artery and vein by a tube.



Langendorff.



Martin's original method.



Bock.

Fig. 30.—Methods of studying the isolated mammalian heart.

1. *Tschilowitch* uses practically Martin's original method, connecting the pulmonary artery with the pulmonary vein by a tube, the course of the blood being: reservoir, jugular vein, right heart, connecting piece, left heart, aorta, and reservoir.

2. *Langendorff* uses only the coronary circulation, introducing the blood into the aorta under pressure, from which it goes through the coronary circulation and flows out of the right heart. The shape of the heart, number and strength of beats, and the number of drops flowing through the right heart may be measured in this way.

3. *Hedon and Arrous* ligate the aorta and vena cava and connect the pulmonary artery and pulmonary vein directly, feeding the heart with its own blood and keeping it alive by artificial methods.

4. *Heymans and Kochmann* connect the aorta of the excised heart with the carotid of a second animal, letting the blood return through a funnel connected with the jugular; or without the use of a funnel, by connecting the pulmonary artery of the excised heart with the jugular of the animal, and tying the other vessels.

III. **Isolated apex preparations, i. e., ganglion-free heart muscle.** *Porter* has succeeded in maintaining rhythmic contractions of isolated strips of the apex of the heart by injecting oxygenated blood under pressure into a branch of the coronary artery supplying it.

The methods of Langendorff and Porter have been criticized as yielding abnormal results, because they leave the cavities of the heart empty. Their results must, therefore, be interpreted cautiously. *Gottlieb and Magnus* (1903) obviate this difficulty by filling the ventricle with a distensible balloon.

Technical References.—Heinz, 1, 184; Meyer and Gottlieb, 202.

EXERCISE II.—(DEMONSTRATION) PERFUSION OF FROG'S HEART

(REPORTER IV, C)

The frog's heart may be perfused either through the sinus venosus and auricles or through the aorta and ventricle.

Technical References.—The various frog methods are discussed in Abderhalden, 3, 329; Kobert, *Intox.*, 1, 177, 193; Tigerstedt, 2.4, 123; Greene, 69.

Experiment 1. (Optional) Demonstration of Williams' Apparatus.—This has been used extensively in pharmacologic work on the frog's heart, as it permits the study of a number of phenomena under a variety of conditions. An artificial circulation is maintained through the ventricle by means of solutions, to which the poisons may be added. The apparatus (Fig. 31) consists of a reservoir and a system of tubes provided with slit

valves (*V* and *V'*) and a two-way cannula. These allow the perfusing liquid to get into the heart (*H*) and to be pumped in a definite direction. The cannula is introduced through the bulbus aortæ into the ventricle and tied. (The apex of the ventricle may be used alone.) Each contraction of the ventricle forces the blood through *V'* into the upright tube, and from here into the reservoir. The relaxation of the heart allows the liquid to enter from *V*. The auriculoventricular valves prevent the blood from coming back into the auricle. The number of drops flowing into the reservoir can be counted, and give an idea of the work done. By raising or lowering the reservoir the intracardiac pressure can be varied;² by applying the screw-clamp beyond *V'* one may introduce resistance; by clamping this tube altogether and opening communication to a small mercury manometer the absolute pressure can be measured and tracings taken. The changes in volume, corresponding to the extent of the excursions, may be read from the millimeter scale, *MS*.

Technical References.—Williams, 1877, *Arch. exp. Path. Pharm.*, 13, 11; Dreser, 1888; *ibid.*, 24, 223; Rothberger, 1907, *Arch. ges. Physiol.*, 118, 353 (Work of Heart).

Experiment 2. (Demonstration) Perfusion of Ventricle by Straub-Fuehner Method.—This consists in introducing a suitable cannula through the aorta into the ventricle of the excised heart.

¹ The valve *V'* should point in the reverse direction. Fresh frog's skin is convenient for these valves.

² A pressure of 200 mm. of water is the optimum.

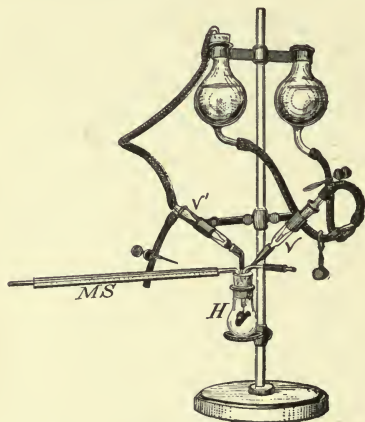


Fig. 31.—Williams' heart apparatus.¹

The preparation may be used to demonstrate the effects of the following drugs:

(a) *Calcium* (Straub, 1912).—A tracing is taken with ordinary Ringer's Fluid. This is then replaced by Ca-free Ringer's: the excursions become very weak. Replace by ordinary Ringer's: the heart recovers. Replace by Ringer's containing CaCl_2 0.8 : 1000 (instead of the normal 0.2 : 1000): the excursions are again diminished. A definite ratio of Ca is therefore necessary for functionation. (The calcium does not penetrate into the muscle, but acts on the cell membrane.) Replace by ordinary Ringer's solution and let conditions return to normal.

Question.—Describe the action of calcium on the heart muscle.

(b) *Potassium.*—Use 0.5 c.c. of 10 per cent. in 10 c.c. of Ringer's.

(c) *Strychnin.*—Use 1 c.c. of 1 : 1000; then 1 c.c. of 1 : 100 in 10 c.c. of Ringer's.

(d) *Caffein.*—Use 1 c.c. of 1 : 100 in 10 c.c. of Ringer's.

(e) *Aconitin.*—Use 1 c.c. of 1 : 10,000 in 10 c.c. of Ringer's.

(f) *Epinephrin.*—Use 1 c.c. of 1 : 10,000 in 10 c.c. of Ringer's.

Questions.—Describe the effects of these drugs. Which are stimulant and which depressant to the heart?

Technical Notes.—The method was described by Straub, 1910, Bioch. Zt., 28, 394, and modified by Fuehner, Nachweiss, 123, as follows:

Large frogs (60 to 100 gm.) should be used. A small dish with Ringer's solution and containing the cannula (Fig. 32) should be at hand; also ligatures and dissecting tools.

Decapitate the frog, leaving the lower jaw, and pith the spinal cord. Lay frog on plate, head toward operator. Lift the skin of the throat with forceps and cut away a wide flap of skin over the thorax, reflecting it down over the abdomen.

Rinse the scissors and split the sternum, from above downward, to the abdominal muscles, where the opening is enlarged by a transverse incision. Cut away the sternum on both sides to the arms. Turn the plate about so as to bring the feet toward the operator.



Fig. 32.—Fuehner's heart cannula, actual size (Fuehner).

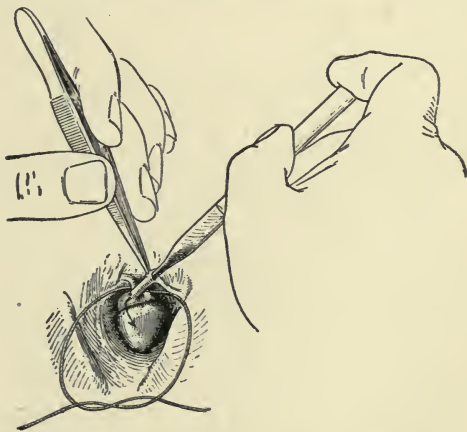


Fig. 33.—Insertion of aortic cannula (Fuehner).

Slit the pericardium to the aorta, and incise the lungs if they interfere. Place a ligature around the aorta beyond its bifurcation and loop for tying, but do not tie. Incise

one of the branches and insert the cannula, containing a little Ringer solution, and very carefully push it into the ventricle in the direction of the arrow in Fig. 33. This is a rather delicate operation. Force must be avoided, the cannula being slipped gently to and fro, toward the back and left side of the frog, until it enters during a systole (practice on dead frog).

The cannula enters rather suddenly, necessitating care that it does not slip back. Tighten the ligature, making sure that the aorta has actually entered by carefully feeling the ventricle and observing the movement of the blood in the cannula. Remove this with a pipet, the point of which should come to, but not enter, the heart (Fig. 34). Rinse with Ringer's until the solution remains blood free. Raise the cannula and excise the heart, dividing successively the aorta, frenulum, and cavæ as far as possible from the sinus. It is rather advantageous to tie the vein before dividing (Fig. 35).

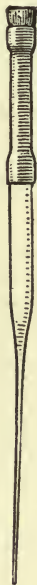


Fig. 34.—Pipet for heart cannula, reduced size (Fuehner).

The apex of the ventricle is now gently clasped with a delicate rather broad-pointed clamp (Fig. 36). (Mendenhall, 1915, Jour. Pharmacol., 6, transmits the movements through a small tambour connected with the top of the cannula.) Fasten the heart-cannula in a moist chamber, through which oxygen is bubbling, and connect for tracings (Fig. 37).

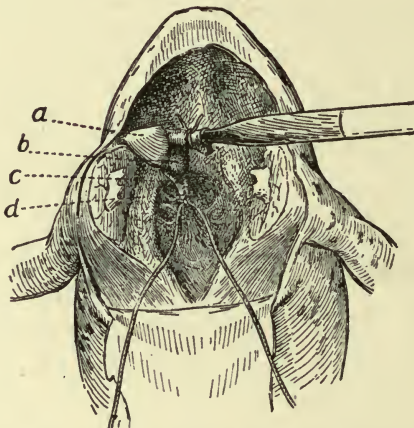


Fig. 35.—Excision of heart (Fuehner): *a*, Ventricle; *b*, auricles; *c*, sinus venosus; *d*, vena cava.

Experiment 3. (Optional) Heart Perfusion in Bio-assay.—The Straub preparation may be used for Digitaloids, Aconitin, and Muscarin (Fuehner, Nachweiss, 128; *Proof of Aconitin*, *ibid.*, 102 and 130; Arch. exp. Path., 1911, 66, 179.

Experiment 4. (Optional) Perfusion of Auricles and Ventricle by Hartung's Method.—Arch. exp. Path. Pharm., 1911, 66, 3.

This maintains a circulation through the entire heart. A. J. Clark, 1912, has introduced some slight modifications, Proc. Roy. Soc. Med., 5, 181. Simpson, 1911, Quart. Jour. Exp. Physiol., 4, 249, describes a cardioplethysmograph. Santesson, 1915, Nord. Med. Ark., prefers perfusion through the vena cava *in situ*.

The apparatus may be used to demonstrate the action of *aconitin* 1 : 100,000.

Experiment 5. (Optional) Perfusion of Frog's Heart in Situ.—The most convenient method consists in placing cannulae into the ascending vena cava and in one of the aortæ, both pointing toward the heart, and ligating the other vessels. The vein cannula is connected by rubber tubing with a Mariotte bottle. Air-bubbles must be rigorously excluded. The aortic cannula is also connected with a tube, through which the fluid can return to the reservoir. The latter is filled with the perfusing fluid—Ringer's solution. By raising the reservoir the diastolic pressure can be varied at will—4 to 6 cm. gives the best results. The resistance to the heart can be varied by raising or partially clamping the aortic tube.

The observations are made by counting the number of beats and the outflow per

minute. Tracings may be taken by any of the methods. The heart may be left in the body or excised.

If drugs are to be perfused, it is well to connect two reservoirs with the vein cannula. Any of the following drugs may be used (Greene): Alcohol, 2 to 5 per cent.; Caffein, 0.1 and 0.2 per cent.; Calcium Chlorid, 0.03 per cent.; Chloral, 0.2 per cent.; Chloroform,



Fig. 36.—Isolated heart (Fuehner).

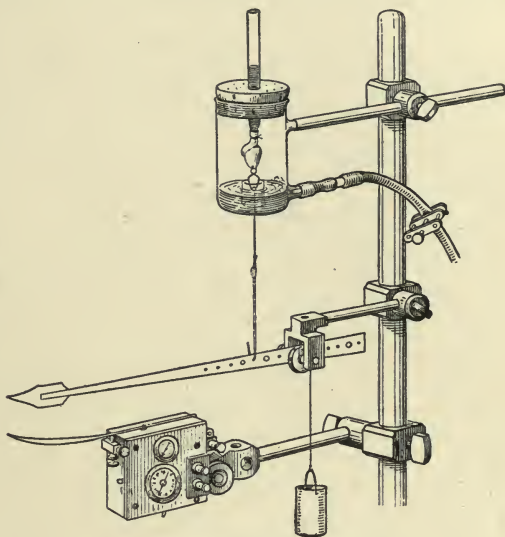


Fig. 37.—Heart-chamber (Fuehner).

0.05 per cent.; Ether, 1 per cent.; Morphin, 0.5 per cent.; Quinin, 0.05 and 1 per cent.; Veratrin, 0.005 per cent.

Experiment 6. (Optional) Isolated Auricle of Frog.—See W. Straub, *Arch. exp. Path. Pharm.*, 79, 19.

EXERCISE III.—BIO-ASSAY OF HEART TONICS: DIGITALIS, ETC.

(REPORTER IV, C)

Introductory Discussion of Bio-assay.—The natural variability of botanic and animal drugs and their deterioration on keeping, etc., necessitate the determination of their strength, especially in the case of potent drugs. Chemic assay of the active constituents when possible is preferred. However, when a drug contains several active constituents, and particularly when these are not identified, chemic assay is not feasible. In such cases the activity may be estimated through comparative experiments on animals, by determining the dose required to produce some definitely ascertainable pharmacologic response. When a drug contains several ingredients producing rather different effects the test should refer to the action which is especially utilized in therapeutics. Because of the variability of biologic reactions the results are not usually as accurate as are the better chemic methods, but they are at least much better than nothing. The methods should, so far as possible, exclude marked personal factors in technic or in interpretation.

The digitalis assays are all based on the cardiac action of the drug. They differ mainly in the convenience of their application.

Technical References.—Pittenger; Fuehner; H. C. Wood, Jr., 1912, Jour. Amer. Med. Assoc., 59, 1433; Philadelphia Commission, 1911, Amer. Pharm. Assoc., Bul. 6, 22; Houghton, 1911, Amer. Pharm. Assoc., Bul. 6, 176.

General Discussion of Digitalis Methods.—Hale, 1911, Hyg. Bul., No. 74; Pittenger; Heinz, 1913, Merck's Rep., 26; Holste, 1914, Zs. exp. Path., 25, 385; Barger and Shaw, 1904, Yearb. Pharmacy; Santesson, 1915, Nord. Med. Ark.

Experiment 1. (Demonstration) Official Frog Method (U. S. P. IX).—Exact graded doses are injected into the ventral lymph-sac of weighed frogs (best between 20 and 30 gm.). These are pithed at the end of one hour (Famulener and Lyons method) or of twenty-four hours (Houghton method). The two methods give very similar results. The heart is exposed and inspected. The end-reaction is definite arrest of the ventricles in systole with the auricles dilated. The dosage which just suffices to produce this effect corresponds to "M. F. D." (minimum fatal dose). With digitalis this should correspond to about 0.6 mg. per gram of frog.

Since this dose varies not only with the sample of the drug, but also with the species of the frogs, the season, and other uncontrollable conditions, the sample to be tested must always be compared with a sample of known activity.

Ouabain (crystallized strophanthin)¹ is used for this purpose. The ordinary M. F. D. of this is about 0.00045 mg. per gram of frog.

For exact work the details of the official process must be consulted. The method may be demonstrated by injecting three weighed frogs (about 20 gm. weight) with a 1 : 50,000 solution of ouabain (1 c.c. = 0.02 mg.), giving respectively 0.25, 0.5, and 0.75 per frog (corresponding to 0.00025, 0.0005, and 0.00075 mg. per gram, respectively). After an hour the frogs are opened. The ventricle should be beating in the animal with 0.25 c.c., arrested in that with 0.75 c.c.; that with 0.5 c.c. may be either beating or stopped.

Dilute some Tr. Digitalis with an equal volume of water, and inject into three frogs, using respectively 0.2, 0.4, and 0.6 c.c. of the dilution per 20 gm. frog, corresponding to 0.5, 1, and 1.5 mg. of Digitalis per gram. From the M. F. D. calculate how much Digitalis corresponds to 0.0005 mg. of Ouabain.

The following doses are accepted as equivalent to the standard dose of ouabain (0.0005 mg. per gram of frog):

Preparation.	Dose (gm. or c.c. per gram of frog).
Digitalis.....	0.0006
Tincture.....	0.006
Strophanthus.....	0.000006
Tincture.....	0.00006
Convallaria.....	0.00018
Apocynum.....	0.00005
Squills.....	0.0006

Question.—Describe the principle of the official Digitalis Assay.

Technical References.—U. S. P. IX; Hale and Service, 1911, Amer. Jour. Pharm., 83, 97; Hale, 1911, Hyg. Bul., No. 74; Hamilton, 1912 (Heart-ionic Unit, H. T. U.), Amer. Jour. Pharm., 84, 97; Houghton and Hamilton, 1909, Amer. Jour. Pharm., Oct.; Houghton, 1909, Lancet, June 19; Rowe, 1915 (Comparison One- and Twelve-hour Method), Jour.

¹ Houghton proposed crystallized kombe strophanthin as standard (Amer. Pharm. Assoc., Bul. 6, 176), but this has not been accepted.

Amer. Pharm. Assoc., 4, 108; Committee, Jour. Amer. Pharm. Assoc., 1912, 1, 1305; Gottlieb, 1914, Muench. med. Woch., 813.

Minimal Fatal Dose.—Houghton, 1909, Lancet, June 19.

Effect of Temperature.—Sollmann, Mendenhall, and Stingel, 1915, Jour. Pharmacol., 6.

Weight Fluctuations in Frogs.—Guthrie and Guthrie, 1914, Soc. Exp. Biol. Med., 11, 144.

Seasonal Changes.—Vanderkleed, 1912, Amer. Jour. Pharm., 84, 14; Central Nervous System, Donaldson, 1911, ref., Zbl. Bioch. Bioph., 12, 599.

Identification of Frogs by Spots.—Hatcher, 1909, Amer. Jour. Pharm., 23, 303.

Probability Curve.—Tigerstedt, 3, 5, 36; *Mathematical Methods in Biology*, Abderhalden, 8, 573.

Experiment 2. (Optional) Focke's Method.—This is based on the acute cardiac death. It is open to the criticism that the time of observation is too short to insure complete absorption. The details are described by Focke, Zs. exp. Path., 14, 262; Fuehner, Nachweiss, 95; Pittenger, 45.

Experiment 3. (Optional) Guinea-pig Method of Reed and Vanderkleed.—This determines the minimum hypodermic dose which is fatal in twenty-four hours per 250 gm. of guinea-pig (for instance, 0.1 mg. of Digitalis). The details are described in Pittenger, 25. The same method may be used for a number of other drugs, and is official for Aconite in the U. S. P. IX (see Experiment 6).

Technical References.—M. F. D. of Cardiac Stimulants and Depressants for Guinea-pigs.—Githens and Vanderkleed, 1910, Amer. Jour. Pharm., 82, 453. *Seasonal Variations*, Vanderkleed, 1912, Amer. Jour. Pharm., 84, 14.

Experiment 4. (Optional) Estimation of Activity on Excised Heart.—The method is adapted to special research problems rather than to routine assay.

References.—Straub, 1910, Bioch. Zs., 28, 395; Mendenhall, 1915, Jour. Pharmacol., 6; Krailsheimer and Schmiedeberg, 1910, Arch. exp. Path., 62, 296.

Experiment 5. (Optional) Cat Method of Hatcher.—This determines the M. F. D. for cats on slow intravenous injection. This "cat-unit" corresponds to 0.1 mg. of ouabain per kilogram. In case of slowly acting digitaloids only a partly fatal dose is given, and the reaction is completed with the ouabain. Details and doses, Hatcher and Brody, 1910, Amer. Jour. Pharmacy, 82, 362; Jour. Amer. Med. Assoc., 54, 1050; Pittenger, 31.

Experiment 6. (Optional) Gold-fish Method of Pittenger and Vanderkleed.—Jour. Amer. Pharm. Assoc., 4, 427, 1915.

Experiment 7. (Optional) Bio-assay of Aconite, 'Official U. S. P. IX Method.—This consists in the determination of the hypodermic dose just fatal to the guinea-pig in twelve hours. The standard dose per gram of pig is 0.00004 c.c. of the Fluidextract, 0.0004 of Tincture.

Technical References.—Fuehner, Nachweiss, 102; Arch. exp. Path., 66, 179. Other bio-methods, including *Squibb's Taste Method*, are described in detail by Ford, Ford and Wine, 1915, Amer. Jour. Phar., 87, 489.

EXERCISE IV.—(ALL GROUPS) EXPOSED FROG HEART

(REPORTER V, C)

Inspection of the exposed heart often reveals certain phenomena, especially irregularities, more satisfactorily than do tracings. The drugs may be applied directly or administered systemically, especially into the lymph-sac of the thigh.

Exposure of Heart of Frog.—Pith, decapitate, or anesthetize the animal by injecting 0.2 gm. of Urethane (2 c.c. of 10 per cent.) into the lymph-sac. In ten to fifteen minutes, when motion is paralyzed, raise a fold of skin with forceps, and cut away a strip, not over 2 cm. wide, over the cardiac region. With scissors divide the center of the sternum from above; the lowest cartilaginous portion is cut somewhat to the left to avoid the median vein. The arms are pulled apart and fixed to a small board with pins. The heart can then be seen beating. If it is to be treated with reagents, the pericardium should be opened. The frog's heart will be seen to consist of two auricles and a single ventricle. From the ventricle arises the small, whitish bulbus aortæ, and from this the two aortæ. If the heart is turned up, it will be seen that the auricles are continued into the sinus venosus. A white line marks the junction of the two. The stimulation of this line stimulates the vagus ganglia. If the heart is to be handled considerably, it will be convenient to place a silk ligature around the frenum, the delicate fibrous band attaching the lower surface of the heart to the pericardium. This can then be divided and the heart turned by the ligature.

Injection of Drugs.—The drugs are injected into the lymph-sac of the left thigh (which is not fastened), inserting the needle about 1 cm. below the knee-joint of the extended leg, and pushing it upward. After the injection is made the knee is flexed to prevent leakage.

Local Application.—Solutions may be applied directly to the heart with a pipet or camel's hair brush. The application should be renewed every five minutes, just after the observations.

Observations.—These should bear on the rate of auricles and ventricle; the size, relative strength, and duration of systole and diastole, the color and regularity. The results should be plotted as curves, as shown in Fig. 38.

Tracings.—These may be taken either by (a) resting a light lever directly on the heart; or (b) by attaching a small piece of cork to the muscle-lever, in place of the weight, and resting this weight on the heart; or (c), by the suspension method, passing a fine thread around or through the apex, and connecting with a muscle-lever; or (d) by connecting one of the aortæ with a small mercury manometer. When levers are used with the heart, they should be light and well balanced.

Technical References.—Fuehner, 90; Heinz, 1, 820; Kobert, Intox., 1, 193; Greene, 69; Tigerstedt, 2, 4, 123.

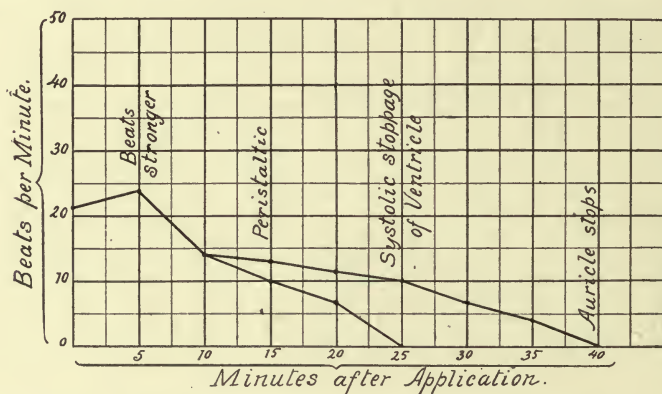


Fig. 38.—Diagram of observations on the effect of digitalis, frog's heart.

Experiment 1. (Group I) Digitalis, Inspection.—Anesthetize frog with Urethane. After ten minutes inject into thigh lymph-sac Tr. Digitalis, 1 c.c. After five minutes expose the heart and continue the observations until the heart stops. Plot curves as shown in Fig. 38. The effects consist in an increased tone of the cardiac muscle; the beats are slowed (sometimes there is a preliminary quickening) and strengthened. The systole particularly increases, the heart becoming progressively smaller and whiter. The contractions then become irregular and often peristaltic. The slowing continues and affects particularly the ventricle, so that there may be several auricular beats in each contraction of the ventricle. Finally the heart stops in systole, *i. e.*, as a small white lump. It may be necessary to apply a 20 per cent. infusion to obtain this result. If the ventricle be distended by injecting 0.75 saline under pressure (with a hypodermic syringe), it will again contract. The application of aqueous camphor solution, or pricking with a needle, starts only a few beats.

(Optional) **Local Application.**—Instead of injecting the digitalis, it may be applied locally as 5 per cent. infusion. Veratrin ($\frac{1}{2}$ per cent.) or BaCl_2 (1 per cent.) give effects very similar to digitalis.

The results are sometimes atypical.

Experiment 2. (Group II) Digitalis Tracing.—Inject Urethane and Digitalis as in Experiment 1; but after exposing the heart, insert a hook in the apex, connect with a heart-lever, and take slow-speed tracings.

(Optional) *Oubain*.—In place of digitalis, in the above experiments, inject ouabain, 0.01 mg. ($\frac{1}{2}$ c.c. of 1 : 50,000).

Experiment 3. (Group III) Aconite Tracing.—Anesthetize frog, expose heart, and take normal tracing. Inject into thigh 20 mg. of aconite (0.5 c.c. of 4 per cent.): successively, increase of rate, cardiac peristalsis, diastolic arrest.

Experiment 4. (Group IV) Aconite, Inspection.—Pith the brain of a frog, explore the heart, open the pericardium, and apply a 4 per cent. infusion of Aconite. Plot curves as shown in Fig. 38.

Aconite stimulates and then paralyzes successively the accelerators, vagus, and muscle. If the results are typical the rate is first quickened, then slowed, then again quickened and irregular, and then gradually slowed, with final paralysis in the median position. The primary quickening may be absent. The secondary quickening is fairly constant and characteristic. The most striking feature is the extreme irregularity and arrhythmia of the heart in the later stages. This may take the most varying forms. The two sides of the ventricle often beat alternately, the blood being pumped from one side to the other.

Experiment 5. (Group V) Comparative Toxicity of Anesthetics.—Excise the hearts of three frogs. Place a heart in each of three watch-glasses containing the following normal solutions: (a) Normal saline; (b) normal saline saturated with chloroform; (c) normal saline saturated with ether. Note that the chloroform stops very quickly, the ether heart much later. The stoppage is in the median (paralytic condition), and is preceded by slowing and weakening. If the hearts are at once removed to normal saline, they may beat again.

The greater toxicity of the chloroform is emphasized by the fact that it is much less soluble than ether, the saturated solution containing only a twentieth as much of chloroform as of ether.

Questions.—(a) Describe the effects of digitalis, aconite, and anesthetics. (b) Is ether or chloroform more dangerous?

EXERCISE V.—OPTIONAL EXPERIMENTS ON FROG HEART

Experiment 1. Chloral and Camphor.—Inject into ventral lymph-sac of frog 40 mg. of chloral (0.4 c.c. of 10 per cent.). In ten or fifteen minutes expose the heart and start tracing. When heart action is weak and slow, irrigate with N. S. (for control), and then with saturated solution of camphor in N. S. The beat is materially strengthened (Böhme, 1905, Arch. exp. Path. Pharm., 52, 347). The stimulation is seen only on depressed frog hearts and not in mammals (Plant, 1905, Jour. Pharmacol., 5).

Experiment 2. Other Drugs for Lymph-sac Injections and Tracings.—Alcohol, 1.5 c.c.; Atropin, 1 c.c. of 1 per cent.; Digitalis, 0.5 c.c. of 5 per cent.; Morphin, 0.5 c.c. of 10 per cent.; Physostigmin, 0.1 c.c. of 10 per cent.; Pilocarpin, 0.6 c.c. of 10 per cent.

Experiment 3. Other Drugs for Local Application and Inspection.—Antipyrin, 1 per cent.; Caffein, 1 per cent.; Chloral, 1 per cent.; Chloroform, 0.5 per cent.; Potassium Chlorid, 0.9 per cent.; Quinin, 0.1 per cent.; Strychnin, 0.01 per cent.

Experiment 4. Lymph Hearts of Frog.—Kobert, Intox., 1, 195.

EXERCISE VI.—(ALL GROUPS) PERFUSION OF TURTLE HEART

(REPORTER I, D)

Technic.—Arrange a Mariotte perfusion bottle (Fig. 39) with about 250 c.c. of Ringer's Fluid, connected with a rubber tube about 25 cm. long, furnished with a pinch-cock and ending in a cannula of about 2 to 4 mm. end diameter, for insertion into the vena cava. Fill the connections with the fluid, have ready another 25-cm. rubber tube, ending in a cannula of 1 to 3 mm. end diameter, for the aorta. The other end of this tube is furnished with a bent glass tube. Fasten the bottle on a stand about 20 cm. above the table.

Draw out the head of the turtle and destroy the brain by a blow with a hammer. Cut through the junction of the lower shell (plastron) with a saw or bone forceps and remove it with a scalpel. Expose the heart and remove the pericardium. The animal can be supported on its back by a towel twisted into a ring. Insert the cannulae into a vena cava and into the aorta and tie all other vessels.¹ Excise the heart.¹

Connect with the perfusion apparatus, avoiding air-bubbles. Fix the cannulae in a clamp so as to support the heart firmly. Place a hook or clamp on the apex of the heart and connect with a lever tracing on slow drum. The level of the perfusion fluid should be about 10 cm. above the heart. Place the free end of the aortic tube in a graduate, about 15 cm. above the heart, and measure the outflow per minute or other convenient period.

Experiment 1. (Group I) Antipyrin and Epinephrin.—Obtain normal tracing and observations. Add Antipyrin² to the perfusion bottle in the proportion of 1 : 4000 (2.5 c.c. of 1 per cent. per 100 c.c. of Ringer's). When the contractions have become very weak, inject slowly with a hypodermic syringe into the vein-tube about 1 c.c. Epinephrin, 1 : 100,000; stimulation.

Experiment 2. (Group II) Aconite and Epinephrin.—Proceed as in Experiment 1, using Aconite, 1 : 500 (2 c.c. of Tincture per 100 c.c. of Ringer's). The heart passes through the peristalsis to a final slowing. Inject Epinephrin as in Experiment 1: stimulation.

Experiment 3. (Group III) Alcohol and Epinephrin.—Obtain normal tracing and observations. Add Alcohol to the perfusion bottle, raising the concentration (with observations and tracings), progressively, through $\frac{1}{2}$, 1, and 5 per cent. ($\frac{1}{2}$, 1, and 5 c.c. per 100 c.c. of Ringer's Fluid): the lower concentration is inactive, the higher produces some depression. Inject Epinephrin as in Experiment 1.

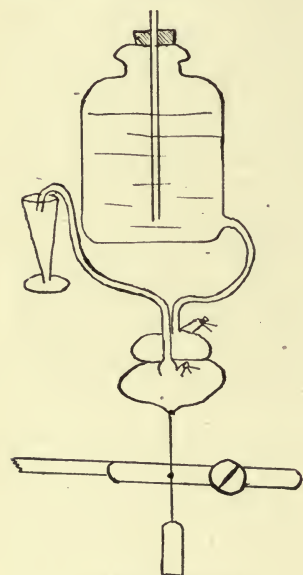


Fig. 39.—Perfusion of turtle heart.

¹ The heart may be left in position. This is more convenient, but becomes disturbing if the animal should move.

² Or Phenol, 1 : 5000 (2 c.c. of 1 per cent. per 100 c.c. of Ringer's).

(Optional) *Physostigmin*, 0.5 c.c. of 1 per cent. per 100 c.c. may be used in place of Epinephrin.

Experiment 4. (Group IV) Potassium and Epinephrin.—Proceed as in Experiment 1, using Potassium Chlorid (5 c.c. of 10 per cent. per 100 c.c. of Ringer's Fluid). When the heart is greatly weakened or arrested, inject Epinephrin as in Experiment 1.

Experiment 5. (Group V) Digitalis and Potassium.—Proceed as in Experiment 1, using Digitalis 1 : 10,000 (0.1 c.c. of Tincture per 100 c.c. of Ringer's). When the heart has gone into systolic standstill, see whether it can be started by raising the pressure in the aortic tube.

Inject into the vein tube 1 c.c. of 10 per cent. KCl. If this does not start the heart, see whether it can be recovered by perfusion with unpoisoned Ringer's Solution.

Questions.—(a) Describe the effects of the drugs.

(b) Is the digitalis standstill due to paralysis of the cardiac muscle? Why?

EXERCISE VII.—(DEMONSTRATION) VAGUS POISONS ON TURTLE

(REPORTER III, D)

Technic.—Destroy the brain of a turtle and remove the plastron, as explained in Exercise VI. Draw out the head, so as to put the neck on the stretch and fasten it in position by a nail. Cut away skin and fascia at base of neck and dissect the vagus nerves: they emerge from the long retractor muscles of the head just posterior to where the coracohyoid muscles join in the median line. In the upper part of its course the vagus lies internal to the retractors, then winds around to the front of these muscles (Edmunds and Cushny, 139). It is accompanied by the sympathetic, from which it can be distinguished by the results of electric stimulation. (The right vagus is much more effective, Garrey, 1911, *Amer. Jour. Physiol.*, 28, 330.) The electrodes may be left in place. Attach a hook to the apex of the heart, attach to a lever, and take normal tracing. (A special turtle myocardiograph is described by Cushny, 1905, *Arch. Intern. Pharmacod.*, 15, 493; Edmunds and Cushny, *Lab'y Guide*, p. 144.) Make the following experiments, taking tracings:

1. Record effect of stimulation of vagus.
2. Paint heart with 0.5 per cent. Pilocarpin. When contractions have become very slow, paint with
3. Atropin, 0.1 per cent.
4. Note that stimulation of vagus is now ineffective.
5. Again paint with Pilocarpin, and note that response to vagus returns more or less perfectly.

Excise the heart, place in 0.75 NaCl, and keep for Exercise IX.

QUESTIONS

- (a) Describe the effect of pilocarpin.
- (b) How is this affected by atropin?
- (c) What effect has atropin on the vagus?
- (d) What light does this throw on the mechanism of the pilocarpin slowing?

EXERCISE VIII.—(DEMONSTRATION) VAGUS POISONS ON FROGS

(REPORTER III, D)

The same experiments can be performed as in Exercise VII, but frogs are less satisfactory, because the vagus trunks sometimes do not respond to the stimulation. Frogs, however, are well suited for studying *vagus ganglia*. These are reached by lifting the heart and stimulating the junction of the auricles and sinus venosus.

Experiment 1.—(a) Pith the brain of a frog, pin on board, expose heart, and remove pericardium. Note that electric stimulation of the sinus venosus stops the heart (stimulation of vagus ganglia).

(b) Apply atropin (1 : 1000): In a few minutes stimulation of the sinus produces no effect (paralysis of vagus endings). The atropin may cause a quickening of the heart by stimulating the muscle.

(c) Wash off the atropin with normal saline. Apply muscarin (1 : 1000) (or physostigmin): sinus stimulation is again effectual, and heart may be slowed (stimulation of vagus endings and cardiac muscle).

(d) Wash with normal saline and repeat (b): same effect. Atropin and muscarin (or physostigmin) have antagonistic actions, and whichever is used in larger quantities can overcome the effects of the other. This holds for all peripheral structures upon which these alkaloids act.

Experiment 2. (Optional) Quantitative Estimation of Muscarin by Excised Heart.—See Fuehner, 1908, Arch. exp. Path. Pharm., 59, 179 (Nachweis, 137).

Technical Notes on Cardiac Nerves of Frog.—The *vagus trunk* comes to the surface at about the angle of the jaw, in company with the glossopharyngeal and hypoglossal

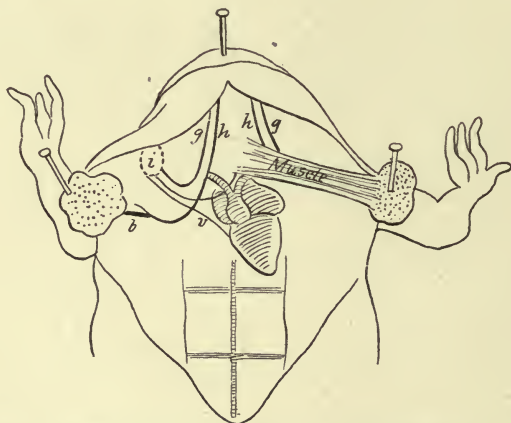


Fig. 40.—Dissection of vagus, frog: v, Vagus nerve; h, hypoglossal nerve; g, glossopharyngeal nerve; b, brachial plexus; j, jaw.

nerves, lying between the two. By exposing this area the vagus can easily be seen passing to the heart (Fig. 40). It may be dissected out and placed on a ligature for stimulation, but frequently it suffices to stimulate it *in situ*.

For the dissection of the *accelerator nerve*, see Stewart's Manual.

QUESTIONS

- (a) Does atropin paralyze the vagus ganglia?
- (b) Where, then, must its action be situated?

(c) Since muscarin or physostigmin act after atropin, where could their action be situated?

(d) Since atropin also acts after these, where must their actions be located?

EXERCISE IX.—(ALL GROUPS) DRUGS ON STRIPS OF TURTLE'S VENTRICLE

(REPORTER I, D)

Use the ventricle of the turtle used in Exercise VII. Grasp the left angle of the base of the ventricle with forceps and cut around the apex to the opposite side. This piece may be cut into two or three strips and attached to a heavy muscle lever, weighted with 1 gm., precisely like a gastrocnemius preparation, keeping it immersed in 0.75 per cent. NaCl. Contractions begin in ten to forty minutes. Take normal tracings, and add the following drugs:¹

Experiment 1. (Group I) Alcohol, successively 2, 5, and 10 per cent. (0.4, 1, and 2 c.c. per 20 c.c. N. S.).

Experiment 2. (Group II) Strychnin, 1 : 10,000 (2 c.c. of 1 : 1000 per 20 c.c. N. S.); after ten minutes, *caffein*, 1 : 1000 (2 c.c. of 1 : 100 per 20 c.c. N. S.).

Experiment 3. (Group III) Ouabain, 1 : 100,000 (2 c.c. of 1 : 10,000 per 20 c.c. N. S.): digitalis action.

Experiment 4. (Group IV) Potassium Chlorid, 1 : 200 (1 c.c. of 10 per cent. per 20 c.c. N. S.). When heart is weakened, add *Epinephrin*, 1 : 20,000 (1 c.c. of 0.1 per cent. per 20 c.c. N. S.).

Experiment 5. (Group V) Calcium Chlorid, 1 : 200 (1 c.c. of 10 per cent. per 20 c.c. N. S.). When heart is weakened, add *Epinephrin*, 1 : 20,000 (1 c.c. of 0.1 per cent. per 20 c.c. N. S.).

QUESTION

Describe the effects of the drugs.

TECHNICAL REFERENCES

Greene, 66.

(Optional) *Other drugs which may be used are:* Atropin, 0.001 and 0.002 per cent.; Barium Chlorid, 0.01 per cent.; Chloral, 0.01 per cent.; Chloroform, 0.05 and 0.1 per cent.; Cocain, 0.1 per cent.; Digitalis, 0.002 and 0.005 per cent.; Ergot, 10 per cent.; Ether, 1, 2, 4, and 6 per cent.; Morphin, 1 per cent.; Nicotin, 0.05 per cent.; Nitrite of Sodium, 0.02 per cent.; Physostigmin, 0.1 per cent.; Pilocarpin, 0.1 per cent.; Veratrin, 0.005 and 0.05 per cent.

(Optional) **Heart of Chick Embryos.**—Eggs are incubated for twenty-four to thirty-six hours, carefully opened, the contents floated in a dish, and the membranes cut away. The heart-beat may be observed in a watch-glass, under the microscope, and drugs applied, etc. The heart at this time does not contain nerves. (Pickering, 1893.)

¹ The preparation, after a normal tracing has been taken, may be immersed in the drug until the effect starts, and then returned to the unpoisoned saline.

CHAPTER XXXVII

AUTONOMIC DRUGS: (A) PUPILS; (B) GLANDS; (C) BRONCHIOLES; (D) ANAPHYLAXIS; (E) EXUDATIVE INFLAMMATION**(A) EFFECTS OF DRUGS ON THE PUPIL**

Introduction.—The iris contains two sets of smooth muscle-fibers, the circular sphincters, and radial dilators (Fig. 41).

The sphincter muscle is innervated by fibers contained in the oculomotor nerve. These terminate around the cells of the ciliary ganglion. From here the fibers pass on as the short ciliary nerve.

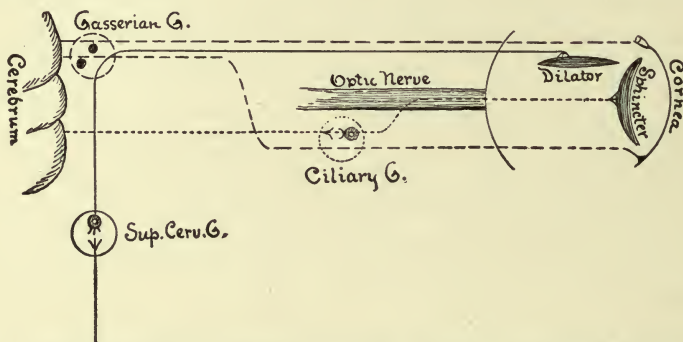


Fig. 41.—Innervation of iris (adapted from P. Schultz): Solid line = sympathetic (dilator); fine dotted line = oculomotor (constrictor); coarse dotted line = trigeminal.

The nerve-fibers for the radial muscles run in the cervical sympathetic nerve, and terminate in the superior cervical ganglion. The fibers which arise here run through the Gasserian ganglion (but without joining any cells), where they unite with the first branch of the trigeminal, and run to the iris in the long ciliary nerve.

The pupils may, therefore, be affected through the following mechanisms:

(A) DILATOR MECHANISM.

1. Sympathetic center.
2. Sympathetic and long ciliary nerve.
3. Superior cervical ganglion.
4. Postganglionic fibers.
5. Endings in radial muscle.
6. Fibers of radial muscle.

CONSTRUCTOR MECHANISM.

7. Oculomotor center.
8. Oculomotor and short ciliary nerves.
9. Ciliary ganglion.
10. Postganglionic fibers.
11. Endings in sphincter muscle.
12. Fibers of sphincter muscle.

Stimulation of "A" causes dilatation; paralysis, constriction through the unopposed action of the constrictor mechanism.

Stimulation of "B" causes constriction; paralysis, dilatation through the unopposed action of the dilator mechanism.

The action may be located as follows (the principal drugs giving these effects are added in parentheses):

A. It is tried whether the drug acts also when applied to the cornea, and if so, whether the effect is confined to this eye, or at least is much greater there. If this is the case, the action must be on the endings or muscle. If the drug acts only when it is introduced systemically, the action must be on the ganglia or centers. The ganglia are discussed below, *Central actions* are usually confined to the dilator center (stimulated by asphyxia, depressed in man by morphin).

B. Dilation of Pupil (Mydriasis).—The oculomotor trunk is exposed and stimulated:
1. No effect. Peripheral constrictor paralysis. It remains to distinguish between the ganglia, endings, and muscle, by stimulation of the short ciliary and of the sphincter muscle. (Atropin paralyzes the oculomotor endings. What would be the result of these stimulations?)

2. Oculomotor stimulation is effective. The dilation must be due to sympathetic stimulation. The drug would be ineffective after section and degeneration of the sympa-

thetic. Stimulation of the ganglia can be shown or excluded by section of the long ciliary. (Cocain stimulates the sympathetic center, ganglion, and endings. Epinephrin stimulates the myoneural function.)

C. Constriction of the Pupil (Miosis).—The cervical sympathetic is stimulated:

1. No effect. Sympathetic paralysis. The distinction between ganglia, endings, and muscle is made by stimulating the long ciliary and the radial muscle. (Nicotin paralyzes the ganglia after a preliminary stimulation.)

2. Sympathetic stimulation is effective. The constriction must be due to oculomotor stimulation. This is generally in the endings (physostigmin, muscarin, pilocarpin). The ganglia may be excluded by section of the short ciliary; the muscle by the fact that large doses of atropin cause dilation.

The localization of these actions requires rather complicated operations; but the local effects and the antagonism can be readily demonstrated.

TECHNICAL REFERENCES ON SPECIAL SENSES

Pupils.—Kobert, *Intox.*, 1, 212, 281; Fuehner, 144.

Subcorneal Inoculation.—Abderhalden, 3, 1285.

Cataract.—Kobert, *Intox.*, 1, 215.

Chemosis.—*Ibid.*, 1, 215.

Iritis and Uveitis.—Guillery, 1915 (prodigious ferment), *Zentr. Bioch. Bioph.*, 18, 71.

Light Sensation.—Tigerstedt, 3.2, 1; *Color sensation*, *ibid.*, 42; *Eye movements*, *ibid.*, 100.

Ophthalmoscopy.—Tigerstedt, 3.3, 55.

Cranial Nerves, Operations.—Tigerstedt, 3.4, 101.

Special Senses.—*Temperature*, Tigerstedt, 3.1, 1; *Pressure*, *ibid.*, 11; *Pain*, *ibid.*, 30; *Odor*, *ibid.*, 46; *Taste*, *ibid.*, 91.

Ear.—*Innervation*, Tigerstedt, 3.3, 181; *Hearing*, *ibid.*, 204; *Acoustics*, *ibid.*, 204.

EXERCISE I.—(DEMONSTRATION) LOCALIZATION OF ATROPIN ACTION ON PUPIL¹

(REPORTER V, B)

Technic.—Anesthetize dog. Tracheotomize. Divide both vagosympathetics and arrange central end for stimulation (right side). Turn right side of heart upward. Make a **L**-shaped incision through skin, the vertical limb from sagittal suture to external canthus;

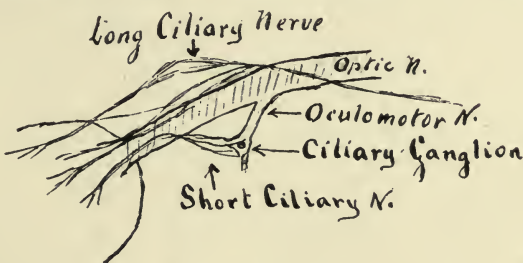


Fig. 42.—Pupillary nerves.

the horizontal limb from internal canthus of right eye along upper border of orbit and whole lower border of zygoma. Stop all hemorrhage. Cut away the upper cartilaginous, orbital border. Open the orbital capsule below the external rectus. Divide and reflect the latter. Carefully clean away fatty tissue until optic nerve is seen. Draw bulbus forward (Fig. 42) and search for place where short ciliaries leave the optic sheath, or search directly for the ciliary ganglion, by drawing the inferior rectus muscle outward and the retractor bulbi upward. Pass threads under the short ciliary and long ciliary nerves. (The long ciliary nerves also run on the optic nerve.)

Confirm the dissection by electric stimulation—the short ciliaries constrict the pupil, the long ciliaries dilate. If there is any difficulty in locating the latter, stimulation of the central vagus may be substituted.

Experiments.—Having confirmed the effects of stimulation, inject a few drops of Atropin (1 : 1000) into the anterior chamber: the pupil dilates.

¹ Jessorow, *Arch. Physiol.*, 1886, 150.

Stimulation of the short ciliary is now ineffective, stimulation of the long ciliary still causes dilation. To show that the sphincter fibers themselves are not paralyzed separate the points of the electrodes to the diameter of the pupil, and thus stimulate, giving a circular motion to the electrodes: the pupil constricts. If the animal is in good condition, inject a few drops of *Physostigmin* (1 : 1000), and see whether the excitability of the oculomotor is restored.

QUESTION

Give the evidence showing that atropin paralyzes the oculomotor endings.

EXERCISE II.—(ALL A GROUPS) LOCAL APPLICATION OF MYDRIATICS AND MIOTICS TO MAMMALIAN EYE

(REPORTER V, B)

General Method.—Drop a few drops of the solution into the eye of the animal with a pipet. Note when the dilatation or constriction sets in—about fifteen minutes (using the other eye for comparison); when it reaches its maximum—about an hour; and when it disappears—about a day. Try whether the light-reflex is preserved. Report the results, stating what conclusions are justified in each case. Cats are best adapted to the study of drugs acting on the pupil. Dogs answer very well. Rabbits can also be used, but are not quite as sensitive. It must also be remembered that in rabbits the two eyes react independently to light, so that the nose of the rabbit must be pointed to the window if the eyes are to be compared. Rabbits do not react to Dionin.

Experiment 1. (Group I, A) Atropin, Pilocarpin, Physostigmin.—(a) Place 2 drops of *Atropin* (1 : 1000) into the eye of the animal: dilation. The effect is confined to one eye. Light-reflex is absent. (Paralysis of oculomotor endings.)

(b) In an hour drop *Pilocarpin* (1 : 100) into the same eye: little effect.

(c) In fifteen minutes drop *Physostigmin* (1 : 100) into the same eye: constriction.

Experiment 2. (Group II, A) Physostigmin.—Into the eye of another animal place 2 drops of *Physostigmin* (1 : 100): constriction confined to the one eye. Appears in fifteen minutes, maximum in about an hour. (Stimulation of oculomotor endings.)

Experiment 3. (Group III, A) Pilocarpin.—Drop *Pilocarpin* (1 : 100) into the eye of another animal: constriction confined to the one eye, but not as great as *Physostigmin*. (Peripheral stimulation of the oculomotor.)

Experiment 4. (Group IV, A) Cocain.—Drop 1 : 100 solution into eye of another animal. Note the anesthesia and dilatation confined to the one eye. The latter is not as strong as with *Atropin*, and the pupils still react to light. (Stimulation of the sympathetic.)

Experiment 5. (Group V, A) Dionin (Ethylmorphin).—Drop some 10 per cent. solution on conjunctiva of dog or cat: hyperemia and edema.

QUESTIONS

- (a) Which of the drugs are mydriatics?
- (b) Which are miotics?
- (c) Which is more powerful, atropin or cocain?
- (d) Which is more powerful, pilocarpin or physostigmin?
- (e) Give evidence showing that the actions are peripheral.
- (f) Describe the effects of dionin.

EXERCISE III.—(ALL B GROUPS) FROG PUPIL

(REPORTER V, B)

Frogs are not generally quite as satisfactory as mammals for the study of pupil changes, but they illustrate some interesting phenomena of antagonism. They are also much more subject to epinephrin and may be used in testing for this drug.

Technic of Excised Eyes.—Pith frog. Insert scissors in mouth and cut off head, except lower jaws. Cut away lower lids. Cut head in two, lengthwise. Pin each piece on cork, cornea straight upward. Cut rings about 3 mm. high from rubber tubing of about same diameter as eye. Place a ring on each eye. This forms a little cup, into which the solution is dropped (Meltzer, 1909, *Deut. med. Woch.*, No. 131).

Experiment 1. (Group I, B) Pilocarpin and Physostigmin.—Prepare the eyes as described. Leave in dark until pupils are dilated. Then apply to one a few drops of 1 per cent. Pilocarpin; to the other 1 per cent. Physostigmin: both are constricted.

Experiment 2. (Group II, B) Atropin, Pilocarpin, Physostigmin.—Prepare the eyes as described. Apply to both a few drops of $\frac{1}{2}$ per cent. Atropin. When pupils have dilated, wash and apply to one 1 per cent. Pilocarpin, to the other 1 per cent. Physostigmin. The pupil of the Physostigmin eye constricts, Pilocarpin does not.

Experiment 3. (Group III, B) Nicotin or Curare, Pilocarpin, Physostigmin.—Prepare both eyes as described. Apply to both 0.1 per cent. solution of Nicotin (or 1 per cent. curare) and leave in the dark. After half an hour wash and apply to one 1 per cent. Pilocarpin, to the other 1 per cent. Physostigmin. Pilocarpin constricts, Physostigmin very little (Dixon & Malden, 1908, *Jour. Physiol.*, 37, 531).

Experiment 4. (Group IV, B) Cocain, Pilocarpin, Physostigmin.—Prepare both eyes as described. Apply to both 1 per cent. Cocain: the pupils dilate. Wash and apply to one 1 per cent. Pilocarpin, to the other 1 per cent. Physostigmin: both constrict.

Experiment 5. (Group V, B) Epinephrin.—(a) Prepare the eyes as described. Apply to both Epinephrin, 1 : 10,000: submaximal dilation. Wash and apply to one Pilocarpin, 1 per cent., to the other Physostigmin, 1 per cent.: both constrict. (Ehrmann, 1905, *Arch. exp. Path. Pharm.*, 53 : 97 (Fuehner, 146) tried to elaborate this reaction into a quantitative method, but Metzer, loc. cit., finds it unsuited to this purpose.)

(b) Pith a frog and inject into lymph-sac 0.01 mg. of Epinephrin (1 c.c. of 1 : 100,000): dilation of pupil.

Further characteristics of the Epinephrin mydriasis are that it is submaximal; the pupils become round; they do not react to light (Metzer, loc. cit.).

QUESTIONS

- (a) Which of the drugs are mydriatics?
- (b) Which are miotics?
- (c) Tabulate the efficiency of pilocarpin and of physostigmin after atropin, nicotin or curare, cocain, and epinephrin.
- (d) Assuming that atropin paralyzes the oculomotor endings, where could the action of pilocarpin and of physostigmin be located?
- (e) How is this limited by the fact that atropin dilates after physostigmin?
- (f) Can the mechanism of the mydriasis by nicotin, cocain, and epinephrin be the same as that of atropin? Why?

(B) EFFECTS OF DRUGS ON (SALIVARY) GLANDS

Introduction.—The peripheral effects of drugs on the iris, on other forms of unstriated muscle, on the vagus mechanism of the heart, and on glands are very similar. The only important exceptions are the muscle of the arterioles and uterus, and the liver, mammary gland, and kidney. The action is the more typical, the more the organ is normally under nervous control.

The more important drugs act as follows:

Pure paralysis of endings: Atropin.

Stimulation of endings: Physostigmin, muscarin, pilocarpin.

Ganglia: These are first somewhat stimulated, and then depressed, by nicotin, coniin, lobelia, spartein, curare, and cocain.

The glandular effects are studied most conveniently on the salivary glands. The submaxillary gland of the dog has the additional advantage that it possesses a double nerve supply. This may be utilized to prove that atropin acts on the endings and not on the gland cells.

Glandular secretion may also be affected through the centers, directly or reflexly. The salivation during apomorphin nausea is an instance of direct central stimulation.

EXERCISE IV.—(OPTIONAL) CHORDA TYMPANI EXPERIMENT

See Stewart's Manual, 450, or Practical Physiology, Beddard, etc., for technic. Insert a cannula in Wharton's duct. Stimulate the cervical sympathetic: the gland becomes pale and secretes a little thick saliva. Stimulate the chorda tympani: the gland flushes and yields abundant thin saliva. Inject intravenously 30 to 40 mg. of *nicotin* for a dog, or 10 mg. for a cat. Stimulation of the chorda is now ineffective, but stimulation at the hilus of the gland (*i. e.*, beyond the ganglion cells) causes secretion. The nicotin has therefore paralyzed the ganglion. Inject 10 to 14 mg. of *atropin* for a dog, 5 to 15 mg. for a cat. Stimulation at the hilus causes no secretion, although the gland flushes. The atropin therefore does not act on the vasodilator endings, but it paralyzes the secretory mechanism somewhere peripheral to the ganglion. Stimulate the sympathetic: this causes secretion. The cells are therefore not paralyzed. The atropin must act on the endings. Inject some 2 per cent. *pilocarpin* into the duct, so that it comes in contact with the cells: secretion resumes, since the pilocarpin stimulation overcomes the atropin paralysis.

QUESTION

State the evidence for the localization of the actions of nicotin, atropin, and pilocarpin.

TECHNICAL REFERENCES

Circulation of Submaxillary.—Tigerstedt, 2, 2, 150.

Distinction of Preganglionic and Postganglionic Fibers (Langendorff, 1892, Cbl. Physiol., 5, 130).—Stimulation of preganglionic becomes ineffective soon after death; the postganglionic remain excitable for one-quarter to three-quarters of an hour (Langley, 1893, Jour. Physiol., 15, 181).

Experiments on Saliva.—Kobert, Intox., 1, 245; Examination, Abderhalden, 3, 257.

EXERCISE V.—(DEMONSTRATION) PILOCARPIN AND ATROPIN ON INTACT ANIMALS

(REPORTER II, A)

The effects may be studied on intact cats or rabbits as follows:

If cats are used, the changes in pulse-rate should also be recorded.

If rabbits are used, the peristalsis may be watched through the abdominal wall.

Experiment 1. Pilocarpin.—Inject hypodermically into rabbit or cat Pilocarpin, 5 mg. per kg. (0.5 c.c. of 1 per cent. per kg.): intense salivation occurs in about half an hour. Peristalsis is greatly increased (diarrhea). The pulse is first slowed, then quickened. The pupils may be constricted. Keep the animal as control for Experiment 2.

Experiment 2. Pilocarpin Antagonized by Atropin.—Inject another animal as in Experiment 1. When salivation is marked, inject Atropin,

10 mg. per kg. (1 c.c. of 1 per cent. per kg.). On comparing the animals after about half an hour, it will be seen that the Atropin has checked the salivation and peristalsis, quickened the pulse, and dilated the pupils. Excitement and forced movements may occur.

QUESTIONS

(a) Describe the effects of pilocarpin on saliva, peristalsis, heart-rate, and pupils.

(b) Which of these effects are antagonized by atropin?

TECHNICAL REFERENCES

Quantitative Antagonism of Pilocarpin and Atropin, Cushny, 1915, Jour. Pharmacol., 6, 439.

EXERCISE VI.—(ALL STUDENTS) REFLEX SECRETION OF SALIVA

(REPORTER II, A)

Place a little dilute acetic acid in the mouth and note the increased salivation. The inhalation of ether acts in the same manner.

TECHNICAL REFERENCES

Pulse-rate of Mammals (A. Reichert, 1909, Bioch. Cbl., 10, 170):

Horse	30-40	Large dog.....	72- 82
Cow	70-85	Small dog.....	70- 90
Ox	52-68	Cat	116-128
Pig	70-86	Rabbit.....	120-140
Goat	70-90	Chicken.....	180-200

Bronchial Secretion.—Henderson and Taylor, 1910, Jour. Pharmacol., 2, 153; J. L. Miller, 1914, Amer. Jour. Med. Sci., 148, 469.

Mucus, Frog Skin.—Kobert, Intox., 1, 189.

Sweat.—Kobert, Intox., 1, 268; *Collection*, human, Abderhalden, 3, 998, 1000.

Milk.—Kobert, Intox., 1, 272.

Secretin: Preparation and Tests.—Abderhalden, 3, 205, 418; 6, 487; 7, 65; Dale and Laidlaw, 1912, Jour. Physiol., 44, XI.

Gastrin.—Preparation, Keetom and Koch, 1915, Amer. Jour. Physiol., 36, 353.

(C) EFFECTS OF DRUGS ON BRONCHIOLES

Introduction.—The bronchial muscles are affected by the autonomic poisons in the usual manner. For instance, they are *constricted* by physostigmin, pilocarpin, and muscarin (stimulation of constrictor endings), and by barium and histamin (direct stimulation of muscle). They are *relaxed* by atropin (paralysis of constrictor endings) and by epinephrin and hordenin (stimulation of dilator endings). Violent constriction occurs in anaphylaxis and in asthma. This may be treated by atropin or epinephrin.

EXERCISE VII.—(DEMONSTRATION) BRONCHIAL TONE IN LIVING ANIMALS

(REPORTER II, A)

This may be estimated by the variation of intrapleural pressure, with constant respiration.

Anesthetize a rabbit. Connect trachea for artificial respiration, interposing an open T-piece for free escape of excess of air. The respiration must be uniform in rate and volume. Cut through cervical cord and destroy respiratory center. Connect jugular vein for injection. Through

a flanged cannula connect one pleura with tambour and take slow tracing of pulmonary excursions. An increase of these excursions is due to dilation of the bronchial muscles, and vice versa.

Inject the following drugs into the jugular vein while taking tracings (the doses are for average animals):

1. *Epinephrin*, 0.1 mg. (1 c.c. of 1 : 10,000): no effect.
2. *Pilocarpin*, 1 mg. (1 c.c. of 1 : 1000): constriction. During this constriction inject:
3. *Epinephrin*, as in (1): relaxation.
4. *Pilocarpin*, as in (2); during constriction inject:
5. *Atropin*, 2 mg. (2 c.c. of 1 : 1000): relaxation.
6. *Histamin*, 0.1 mg. (1 c.c. of 1 : 10,000): constriction.
7. *Atropin*, as in (5); then *Epinephrin*, as in (1): no relaxation.

QUESTIONS

- (a) Which drugs constrict, and which relax, the bronchi?
- (b) What essential difference is there between pilocarpin and histamin?
- (c) What light does this throw on the site of their action?

TECHNICAL REFERENCES

Methods involving the same principles are described by Dixon and Brodie, 1903, Jour. Physiol., 29, 97; Golla and Symes, 1914, Jour. Pharmacol., 5, 92; D. E. Jackson, *ibid.*, 4, 7, 59; 5, 479.

EXERCISE VIII.—(DEMONSTRATION) TREATMENT OF BRONCHIAL SPASM IN PERFUSED LUNG

(Method of Baehr and Pick, 1913, Arch. exp. Path. Pharm., 74, 41.)

(REPORTER II, A)

A guinea-pig of about 250 gm. is etherized lightly. Insert a tracheal cannula, connected through a T-tube, with one limb open, with a respiration bellows of uniform action. Remove sternum. Tie a cannula into pulmonary artery, pointing toward lung. Connect through a T-piece with two perfusion bottles, one filled with glucose-free Tyrode solution, the other with 1 per cent. Witte Peptone in Tyrode. Tie a cannula into the apex of the ventricle for the outflow of fluid (this may be measured if it is desired to study the vascular action). It is best to leave the whole preparation in the thorax. (The excursions of the lung can be recorded by a lever.)

Adjust the perfusion bottles about 30 cm. above the lung, and start the perfusion with Tyrode's fluid. Change to the peptone: the excursions diminish promptly, the lungs remaining rigidly distended, due to bronchial spasm. The condition is analogous to anaphylaxis or asthma.

Change to the plain Tyrode fluid, to which 0.05 per cent. atropin (5 c.c. of 1 per cent. per 100 c.c.) has been added: the spasm is promptly relieved, the lungs returning to their normal volume and excursions.

(The following drugs may be used. In place of Peptone: Histamin, 1 : 100,000; Pituitary, 4 per cent. of the fluid; Pilocarpin or Physostigmin, 1 : 10,000. In place of Atropin: Epinephrin, 1 : 100,000; other drugs are described in the original paper.)

QUESTION

What drugs would be efficient against the spasmodic attacks of asthma?

EXERCISE IX.—(OPTIONAL) REACTIONS OF EXCISED TRACHEAL MUSCLE

(See Trendelenburg, 1912, Arch. exp. Path. Pharm., 69, 106.)

(D) ANAPHYLACTIC REACTION

Introduction.—The injection of proteins sensitizes animals toward subsequent injections of the same protein. The phenomena differ quantitatively in different animals. In guinea-pigs the most conspicuous effect is a bronchial spasm, analogous to that produced by peptone, histamin, pilocarpin, etc.

TECHNICAL REFERENCES

Methods of Anaphylaxis.—Pfeifer in Abderhalden, 5, 525; Zinsser, Hopkins, and Ottenberg, p. 182. For shock-sensitization of dog, 5 c.c. horse serum, hypodermic; after twenty-one days about 5 c.c. by vein (Pearce and Eisenberg, 1910). Dog to egg-white, Edmunds, 1913, Zs. Immun., 17, 127.

Preparation of Protein Poison (from Egg-albumen).—Vaughan and Wheeler, Jour. Lab. Clin. Med., 1, 55, 1915.

EXERCISE X.—(DEMONSTRATION) ANAPHYLAXIS IN GUINEA-PIG; PREVENTION OF ANAPHYLACTIC EMPHYSEMA BY ATROPIN

(REPORTER IV, A)

Two guinea-pigs are sensitized two weeks previously by hypodermic injection of 0.1 c.c. of horse-serum. On the day of the demonstration one of the animals (B) receives 3 mg. of atropin hypodermically, at least ten minutes before the demonstration.

Etherize the atropin pig (B) lightly. Expose the jugular vein. Lighten the anesthesia. With a syringe inject 2 c.c. of horse serum into the vein. Tie vein and remove anesthetic.

Do the same to animal (A). In a very few minutes the animal (A) becomes excited, dyspneic, and dies of asphyxia, usually within five minutes. Open the thorax and note that the lungs are rigidly distended (Auer and Lewis, 1909, Jour. Amer. Med. Assoc., 53, 6).

The atropin pig shows little or no effect. Kill (with chloroform), open thorax, and note that lungs are normally collapsed (Auer, 1910, Amer. Jour. Physiol., 26, 439).

QUESTIONS

- (a) Define anaphylaxis.
- (b) How is it produced?
- (c) What is the essential phenomenon in guinea-pigs?
- (d) Explain how this is relieved by atropin.

EXERCISE XI.—(OPTIONAL) ANAPHYLAXIS IN EXCISED UTERUS

(Dale, 1913, Jour. Pharmacol., 4, 167.)

Sensitize a young virgin guinea-pig with 0.1 c.c. of horse serum fourteen days previously. Kill by blow on head, sever neck, collect blood, and let it clot. Cut across abdomen and perfuse aorta with 500 to 1000 c.c. of Locke's solution to free uterus of serum. Transfer uterus to 200 c.c. oxygenated Locke's solution warmed in bath, connect with levers, and take slow tracing (see Chapter XXXIV, Exercise VIII).

1. Add to the solution successively 0.5 c.c. of various foreign non-specific sera—cat, dog, sheep, ox, etc.: no effect.

2. Add horse serum so as to give a concentration of 1 : 5,000,000 (0.4 c.c. of 1 : 10,000 per 200 c.c.): no effect. Raise the concentration to 1 : 1,000,000 by adding further 1.6 c.c. of 1 : 10,000: some stimulation.

3. Change the Locke solution, obtain normal tracing, and add horse serum 1 : 1000 (0.2 c.c. of undiluted serum): maximal contractions.

4. Again change the Locke solution, obtain tracing, and again add 0.2 c.c., then 1 c.c. of horse serum: no response (desensitization by the dose given in (3) equivalent to anti-anaphylaxis).

5. Change Locke's solution, obtain tracing, and add 4 c.c. of horse serum: contraction.

6. Change Locke's solution, obtain tracing, and add 4 c.c. of the guinea-pig serum: contraction.

QUESTIONS

- (a) Does the anaphylactic sensibility induced by the injections of a specific serum reside in the blood or in the tissues? (Experiment 2.)
- (b) Is the reaction of the tissue confined to the antigen? (Experiments 1 and 2.)
- (c) Under what circumstances does desensitization occur? (Experiments 3 and 4.)
- (d) Is the desensitization specific to antigen or does it apply to all sera? (Experiments 5 and 6.)
- (e) Is the anaphylactic reaction qualitatively or only quantitatively different from the reaction to ordinary serum? (Experiments 4, 5, and 6.)

(E) EXUDATIVE INFLAMMATIONS

These are somewhat allied to anaphylaxis; at least, local exudates are among the phenomena of anaphylaxis. These are markedly influenced by calcium, perhaps because this lessens cell permeability.

Suppuration involves positive chemotaxis and cell necrosis. It is produced by bacterial and certain vegetable proteins; turpentine oil; mercurials; croton oil; or 5 to 10 per cent. silver nitrate.

EXERCISE XII.—(DEMONSTRATION) CALCIUM ON DIONIN CHEMOSIS

(REPORTER IV, A)

Inject cat in morning hypodermically with Calcium Lactate, 20 mg. per kg. (1 c.c. of 2 per cent. per kg.). In afternoon drop some 10 per cent. Dionin in eye: no result. Compare with Exercise II, Experiment 5. (Chiari and Januschke used a drop of mustard oil. Analgesics also influence the reaction, Januschke, ref. Jour. Amer. Med. Assoc., 61, 522.)

EXERCISE XIII.—(OPTIONAL) PREVENTION OF PLEURAL EFFUSION BY CALCIUM

(Chiari and Januschke, 1910, Wien. Klin. Woch., 23, No. 12; 1911, Arch. exp. Pharm. Path., 65, 122.)

About twenty-four hours before the demonstration inject intravenously into two lightly etherized dogs sodium iodid, 1 c.c. of 10 per cent. per kg. One dog (A) serves as control. The other (B) receives at once, hypodermically, calcium lactate, 2 c.c. of 1 per cent. per kg. This dose is repeated in six to twelve hours. In twenty-four hours the dogs, if not already dead, are killed with chloroform, and thorax is opened: the control dog (A) shows abundant pleural exudations, sometimes pulmonary edema and hydropericardium (Boehm, 1876, Arch. exp. Path. Pharm., 5, 329). The calcium dog (B) is dry. (Thio-sinamin, 0.13 gm. per kg. by vein, may be substituted for the sodium iodid.)

QUESTIONS

- (a) What effect has calcium on inflammation?
- (b) How may this be explained?
- (c) Could calcium be useful in serum rash, etc.?
- (d) Suggest why it is of little use in clinical pleuritic effusions.

EXERCISE XIV.—(OPTIONAL) SUSCEPTIBILITY OF CAT'S SKIN TO CROTON OIL

This is increased by feeding with acid, diminished by Ca (Luithlen, 1911, Wien. Klin. Woch., No. 20). The effect of the local application of magnesium sulphate and calcium chlorid could also be tried.

O. Loeb and Loewe, 1916, Ther. Mon., 30, 74, advocate young pigs for experiments with cutaneous irritants.

EXERCISE XV.—(OPTIONAL) SCARLET RED

Inject an oily solution under the skin of a rabbit. This causes epithelial proliferation —although not cancer (B. Fischer, 1906).

EXERCISE XVI.—(OPTIONAL) EXPERIMENTAL PLEURISY

Pleurisy with fibrinous exudate may be produced in dogs by injection of 1 c.c. of oil of turpentine into the pleural cavity (Opie, 1907, Jour. Exp. Med., 9, 391; 1908, *ibid.*, 10, 423). A leukocytic exudate is obtained in rabbits by the intrapleural injection of 10 c.c. of 5 per cent. aleuronat suspension in 3 per cent. starch paste. The animal may be killed and examined after twenty-four hours.

TECHNICAL REFERENCES

Permeability of Vessels.—Estimation by passage of iodid or ferrocyanid into peritoneum, Luithlen, 1913, Med. Klin., No. 42, p. 4.

Differentiation of Exudates and Transudates.—Acetic Acid Test, Barberio, 1914, Zentr. Bioch. 17, 450.

CHAPTER XXXVIII

FATE OF DRUGS; IDIOSYNCRASY; EMETICS. (A) ABSORPTION; (B) EXCRETION; (C) DISTRIBUTION AND INTERACTION OF DRUGS; (D) IDIOSYNCRASY; ATROPIN THYROID TEST; (E) EMETICS; (F) ANTEMETICS.

(A) THE ABSORPTION OF DRUGS

Introduction.—Most drugs must be absorbed before they can produce any action. This holds particularly for drugs which act *systemically*, *i. e.*, on the body cells (in contradistinction to the *locally* acting drugs, the effects of which are confined to the place where they are applied, or to reflexes originating from this point). The subject of absorption has therefore a great practical importance. Absorption may occur from most of the surfaces of the body, but with very different facility. The *intact skin* of mammals is almost impermeable to watery solutions, but absorbs oils and volatile substance. The skin of frogs, however, absorbs watery solutions readily, being rather analogous to mucous membranes. In mammals the most usual channels of absorption are the alimentary canal, the subcutaneous and muscular tissue, and the lungs. The rapidity of absorption varies with the nature of the drug and the place of administration. It is generally proportional to the volatility and solubility of the drug. Volatile substances are absorbed most rapidly from the lungs; watery solutions from intramuscular and subcutaneous injections; resins and oils from the intestinal tract. The absorbability from the different portions of the alimentary canal varies for different animals and drugs. It is generally most effective from the small intestine; less so from the stomach and rectum. The uninjured urinary bladder is practically impermeable, while the mucosa of the urethra is a good absorbing surface. Most mucosæ absorb readily.

Absorption is retarded by the presence of fats or colloids, gums, proteins, or "extractives."

The doses in the comparative experiments must be calculated and measured very accurately. The injection syringe must be washed with a little water, which is then also injected.

TECHNICAL NOTES

Stomach-tube.—This consists of a stout, soft gum catheter (No. 10, English scale, for dogs), attached to the injection bulb shown in Fig. 43.

The mouth of the animal is held open with a perforated gag, the head of the animal is bent forward, and the moistened catheter is passed well back, when no difficulty will be found in making it enter the esophagus. Care must be taken not to push it into the trachea, and it is well to note that the animal does not breathe through the catheter. The accident may also be discovered by the fact that the catheter cannot be pushed as far and that the fluid flows in with much greater difficulty. After making sure that the tube has entered the stomach the solution is poured into the bulb. If it does not flow readily, it can be quickened by blowing.

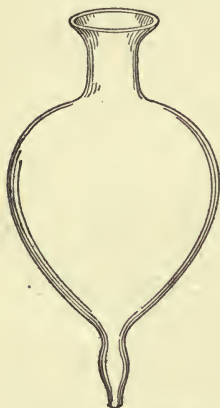


Fig. 43.—Injection bulb (made of a capacity of 100 c.c.).

Perforated Gag.—This is shown in Fig. 44. It is made of hard wood of various sizes. The up-rights are stiff wire rods, to prevent the animal from turning its head. A copper wire may be attached to one rod, brought behind the animal's ears, and wound around the other rod, thus keeping the gag in place.

The *administration per rectum* is done with the same form of apparatus as is used in the stomach. The catheter should be introduced as high as possible. The anus is then closed with bulldog forceps.

Hypodermic injections are generally made under the loose skin of the flank, the animal being held securely. The volume of fluid should be kept below 1 c.c. for guinea-pigs, and below 5 c.c. for dogs. If it is necessary to inject larger quantities, they should be given in fractions, distributed over several parts of the body. The injection of irritant substances should be avoided.

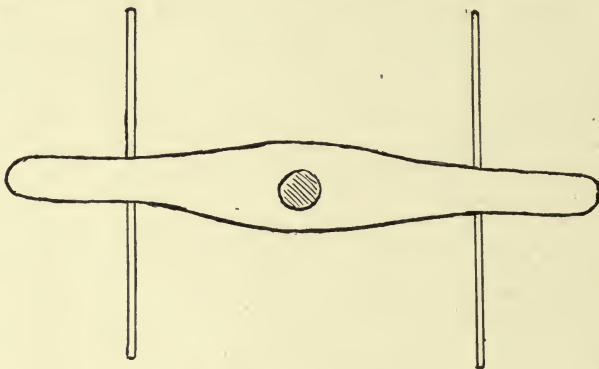


Fig. 44.—Perforated gag.

With dogs and cats the injection is usually made in the back or flank; with rabbits and guinea-pigs, in the abdomen; with rats and mice, at the root of the tail.

An ordinary (1 c.c.) *hypodermic syringe* and strong "antitoxin" needle answers for the smaller quantities; a 5-c.c. antitoxin syringe with an "aspirator" needle is used for dogs.

Intramuscular injections are generally made into the gluteal muscles. *Intraperitoneal* and *intrapleural injections* are made by thrusting in the small needle perpendicular to the surface of the body. In making an intraperitoneal injection the skin and muscles are pinched in the median line below the umbilicus.

For **intravenous injections** a cannula is tied into a vein, pointing toward the heart, and this is connected with a buret containing the solution. The rubber connection should be short to avoid dead space. It is closed by a Mohr clamp. If the injection is to be made slowly, a screw-clamp must be placed on the rubber tube. The greatest care must be used to avoid the entrance of air bubbles into the vein. Before connecting, the rubber tube should be completely filled with the solution, and the cannula should also be filled (with a pipet). If the volume of the injected fluid is small, it may be introduced at air temperature; if it exceeds 10 c.c., it should be brought to body temperature. (Greene advises to surround the buret with the jacket of a Liebig condenser, through which water of the desired temperature is circulated.)

The arrangement shown in Fig. 45 may be used when large quantities of warm fluid are to be infused in long experiments.

If a number of small injections of different drugs are to be made in quick succession, it may be more convenient to clamp the rubber tube $\frac{1}{2}$ inch above the cannula, and to make the injection with a hypodermic syringe, thrusting the needle obliquely through the rubber into the cannula.

The injections may be made either into the femoral or jugular vein. The former is preferred, as the jugular injection introduces complications by bringing the drug directly into the heart in too concentrated a form. It may be necessary in small animals in which it is difficult to introduce a cannula into the femoral vein.

In *unanesthetized rabbits* intravenous injections may be made by thrusting the needle of the hypodermic syringe into one of the ear veins, which has been previously rubbed with xylol and distended by pressure.

Injections into arteries require some pressure. This may be obtained by connecting the top of the syringe with a pressure bottle; or, more conveniently, with the compressed oxygen tank.

Small quantities (1 c.c.) may be injected with an ordinary syringe into the central end of the femoral artery. The action is slower than with intravenous, and more exact than with hypodermic, administration (Mayor, 1908, *Ther. Mon.*, Mch.).

Technical References.—*Stomach-tube.*—Abderhalden, 3, 123; 5, 120; *guinea-pig*, *ibid.*, 3, 1283.

Peritoneal Injection.—Abderhalden, 3, 1286; *Subcorneal*, *ibid.*, 1285.

Intravenous Injection.—Pittenger, 125.

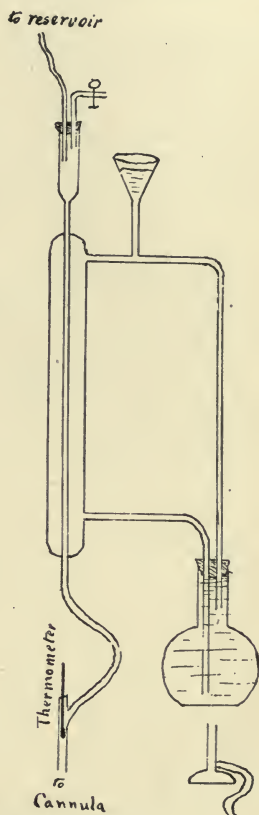


Fig. 45.—Arrangement for prolonged infusion of warm fluids.

Injection into Rabbit's Ear.—Ibid., 3, 1186; 5, 23.

Infusion Under Constant Velocity.—W. Straub, 1911, Muench. Med. Woch., No. 28; Sansum, Wilder, and Woodyatt, 1916, Proc. Amer. Soc. Biol. Chem., 3, 19.

Inoculation of Precise Amounts.—Rosenau, 1904, Hyg. Lab. Bul. No. 19.

Intestinal Absorption.—Kobert, Intox., 1, 252.

Syringes.—Abderhalden, 3, 1278; Pittenger, 121.

EXERCISE I.—(DEMONSTRATION) RAPIDITY OF ABSORPTION BY VARIOUS CHANNELS: EPINEPHRIN AND STRYCHNIN

(REPORTER V, D)

The evanescent action of epinephrin makes it particularly suitable for illustrating this subject. Employing uniform doses, the height of the blood-pressure rise varies directly, the duration of the rise inversely, to the rapidity of absorption.

Experiment 1. Epinephrin.—Arrange an anesthetized dog for blood-pressure tracing; divide both vagi; connect central end of femoral artery and femoral vein for injection. While taking slow tracings, inject uniform doses of epinephrin, viz., 0.05 mg. per kg. (0.05 c.c. or one drop of 1 : 1000 per kg.), as follows, always waiting with the next injection until the blood-pressure has returned to normal:

- (a) Intravenously.
- (b) Under mucosa of nasal septum.
- (c) Into central end of femoral artery.
- (d) Intramuscular.
- (e) Into peritoneum.
- (f) Into vein.
- (g) Into pleura.
- (h) Under skin.
- (i) Into stomach.
- (j) Into vein.

Experiment 2. Strychnin.—Tie the vesico-urethral orifice; inject into bladder a twice fatal dose of strychnin (1.5 mg. \times kg.); in fifteen minutes repeat into ligated stomach; in ten minutes repeat into intestines.

QUESTIONS

- (a) Describe the effects of epinephrin on absorption.
- (b) State the order of its absorbability by the various channels.
- (c) Are all mucous membranes suited to the absorption of strychnin?
- (d) Why was it necessary to tie off the bladder?

EXERCISE II.—(DEMONSTRATION) RAPID ABSORPTION FROM ORAL ADMINISTRATION: NICOTIN AND HYDROCYANIC ACID

(REPORTER V, D)

While most drugs are absorbed relatively slowly when given by mouth, the absolute rapidity varies greatly. With nicotine and cyanid the absorption is almost instantaneous.

Experiment 1. Nicotin.—Place 1 drop of nicotine on the gums of a cat (or 2 drops for a dog). Note heart-rate and time of evidence of the following symptoms: Excitement; salivation; retching; hyperpnea; prostration; convulsions; erection of hairs; arrest of respiration; arrest of heart.

Experiment 2. Hydrocyanic Acid.—Inject 2 per cent. of prussic acid into mouth of rabbit¹ or cat (1 c.c.) or dog (5 c.c.). Observe as for nicotine in Experiment 1. The effects are very similar, but the heart-rate is not quickened and the mucous membranes may not be cyanotic.

¹ Use rabbit of Exercise VIII.

QUESTIONS

- (a) Describe the symptoms of poisoning by (1) nicotine, (2) hydrocyanic acid.
- (b) How soon do they appear with (1) and (2)?
- (c) How rapidly are they fatal with (1) and (2)?
- (d) Which stops first, heart or respiration, with (1) and (2)?
- (e) What difference is there between the color of the mucosæ with the two poisons?
- (f) How is this explained?
- (g) What symptomatic treatment would you suggest for these poisons?
- (h) Discuss its probable efficiency.

EXERCISE III.—(DEMONSTRATION) RAPID ABSORPTION OF GASES BY LUNGS

(REPORTER V, D)

The extensive surface of the alveolar capillaries insures rapid absorption of vapors, provided that they reach the alveoli, and that the epithelium is not impermeable to them.

Experiment 1. Carbon-monoxid Poisoning.—Place a guinea-pig, rat, or other small mammal under a bell-jar and pass coal-gas into the jar: the animal shows almost immediately signs of asphyxia; uneasiness; inco-ordinated convulsions (medullary type); coma; dilated pupils. The mucous membranes, however, are not cyanotic. Remove from bell-jar and start artificial respiration: prompt recovery.

QUESTIONS

- (a) Describe the symptoms of coal-gas poisoning.
- (b) Why are the effects so rapid?
- (c) Which constituent of the gas is mainly responsible?
- (d) How does it act?
- (e) How do the effects differ from those of ordinary asphyxia by oxygen deprivation?
- (f) What should be the treatment for poisoning by the asphyxiant gas?
- (g) What would be the advantage, if any, of administration of oxygen?

EXERCISE IV.—(GROUP I) ABSORPTION OF STRYCHNIN FROM ORAL AND HYPODERMIC ADMINISTRATION

(REPORTER V, D)

Give to a rabbit (A) 1.0 mg. per kg. of *strychnin* sulphate (1.0 c.c. per kg. of $\frac{1}{10}$ per cent.) hypodermically, and to another rabbit (B) the same amount by the stomach-tube. The first rabbit shows the typical strychnin convulsions; the second shows very little effect. Draw a sketch of the typical tetanic condition.

QUESTIONS

- (a) Describe the strychnin symptoms, their onset and duration.
- (b) Would a fatty or resinous substance also be absorbed more readily from hypodermic than from oral administration? Why?
- (c) Would it be probable that a definite ratio between hypodermic and oral dosage could be established that would be valid for all drugs?

EXERCISE V.—(GROUP II) ABSORPTION OF CHLORAL FROM ORAL AND RECTAL ADMINISTRATION

(REPORTER V, D)

Use two weighed cats. Administer to (A) chloral, 0.25 gm. per kg. (2.5 c.c. of 10 per cent. per kg.), by stomach-tube; to (B) the same dose by rectum. Note onset of symptoms—drowsiness, ataxia, anesthesia, etc. Compare temperature and respiration at the end of the experiment.

QUESTIONS

- (a) Describe the symptoms of chloral poisoning.
- (b) Which is the more efficient channel of absorption?
- (c) Would there probably be a constant ratio for all drugs?

EXERCISE VI.—(GROUP III) COLLOID ON ABSORPTION (STRYCHNIN AND STARCH)

(REPORTER V, D)

Use two weighed cats. Administer by stomach-tube to cat (A) strychnin, 1 mg. per kg. (1 c.c. of 1 : 1000 per kg.), diluted with 10 parts of water. Administer to cat (B) the same dose, but diluted with 10 parts of 25 per cent. acacia. Compare the onset and severity of the convulsive symptoms.

QUESTIONS

- (a) Describe the strychnin symptoms.
- (b) What is the influence of the colloid on absorption?
- (c) Would a given quantity of strychnin be as active when given in the form of tincture of nux vomica as if it were given pure?
- (d) Would acacia or starch paste be of any value in strychnin or similar poisoning?
- (e) What would be its limitations?

(B) THE EXCRETION OF DRUGS

Introduction.—Drugs may be excreted by various channels: gases and volatile drugs are excreted mainly by the lungs; metals by the intestinal cells; most substances, however, especially salts and alkaloids, are excreted in greatest quantity by the urine. The saliva, bile, skin, and milk may also aid in excretion; but generally these play a very subordinate rôle.

A knowledge of the excretion of drugs has considerable practical importance; it teaches how frequently the drug must be administered to maintain a continuous action; it also indicates how to hasten the elimination of poisons.

The elimination of drugs by the urine and saliva was studied in Exercise XV. This should be reviewed.

EXERCISE VII.—(DEMONSTRATION) PULMONARY EXCRETION (H_2S)

(REPORTER I, A)

Hold a paper saturated with lead acetate before the nostrils of a rabbit or cat; note that the paper is not blackened; now pass some H_2S into the rectum: the paper becomes blackened (the H_2S being absorbed from the rectum and excreted by the lungs). If the dose of H_2S has been excessive, the rabbit may show paralytic and convulsive effects. (The experiment is

not quite conclusive, for the gas might have reached the paper through the esophagus.)

Not all gases, however, are capable of excretion by the lungs; for instance, ammonia is not excreted by an uninjured lung.

(C) DISTRIBUTION AND INTERACTION OF DRUGS

Introduction.—The distribution of drugs within the body follows special laws, differing for individual drugs. It is also affected by disease, as illustrated by the use of fluorescein in ophthalmic diagnosis. Several drugs may react within, as well as outside of the body, as shown by the calomel-iodid experiment.

A considerable number of drugs undergo chemic changes during their sojourn in the body, being oxidized, reduced, hydrolysed, combined, etc. In some cases the substance is absolutely destroyed. Alcohol, for instance, is almost completely oxidized to carbonic acid and water. In other cases the changes are not so profound. The benzol ring tends to remain intact, but the transformation of acetanilid into paramidophenol illustrates the changes which occur in the side-chains. Benzol derivatives are further excreted as paired compounds, with sulphuric and glycuronic acid.

EXERCISE VIII.—(DEMONSTRATION) IODID, MORPHIN, CALOMEL, FLUORESCIN

(REPORTER I, A)

A rabbit has received, two hours before the demonstration, 50 c.c. of 1 per cent. sodium iodid by stomach-tube and 20 mg. of morphin per kg. (0.5 c.c. per kg. of 4 per cent.) hypodermically. An hour before the demonstration some calomel was dusted on the conjunctiva of one eye. Calomel is also applied to an eye of a normal rabbit.

The animal will present the symptoms of morphin poisoning. The iodid as such produces no visible effects; but the calomel on the eye of the iodid rabbit shows intense congestion and edema, and probably the yellow color of mercuric iodid. The calomel has produced no effect on the eye of the normal rabbit.

The eyes are washed. Into those of the iodid rabbit is dropped some fluorescein solution (fluorescein, 2; sod. bicarb., 3; water, 100). This is left for two minutes, and the eyes are then rinsed with water. Any lesions of the cornea will be stained yellow, while normal tissue remains unstained.

(The animal shall be killed before it recovers from the morphin.)

QUESTIONS

- (a) Describe the effects of morphin.
- (b) Why is it dangerous to use calomel with iodid?
- (c) Describe the fluorescein test.
- (d) Why does fluorescein stain the ulcerated corneas and not the normal?

EXERCISE IX.—(OPTIONAL) DISTRIBUTION AND EXCRETION OF HEXAMETHYLENAMIN

Anesthetize a dog with morphin and ether. Place cannula into one ureter. Administer hexamethylenamin, 0.5 gm. per kg., dissolved in water, by stomach-tube.

Let the urine flow into test-tube containing bromin water, and note time when turbidity first appears corresponding to the beginning of the excretion of the hexamethylenamin.

At fifteen-minute intervals test for presence of hexamethylenamin in urine, blood, and saliva (see page 70). Note the relative intensity of the reaction.

After two to four hours kill the animal and collect the bladder urine, the bile, the pleural, peritoneal, cerebrospinal and synovial fluid, and the aqueous humor. Apply tests for hexamethylenamin and for free formaldehyd. Formulate conclusions.

QUESTIONS

- (a) In what situations is the hexamethylenamin found?
- (b) How soon does it appear?
- (c) What is its relative concentration?
- (d) Where is formaldehyd formed from it?

TECHNICAL NOTES AND REFERENCES ON CEREBROSPINAL FLUID

Continuous Collection of Cerebrospinal Fluid (Dixon and Halliburton, 1913, Jour. Physiol., 47, 218): "The skin at the back of the neck and about an inch from the occipital process is severed for about 1 cm.; the sub-cerebellar cisterna is then punctured by means of a trocar and wide cannula, shaped in the usual way, but with a blunt point. The easiest way of performing this is to flex the animal's head as far as possible and insert the trocar with its point directed to a spot midway between the eyes: it should then pierce the occipito-atlantoid ligament and enter the foramen magnum. The forward movement of the trocar should cease as soon as active resistance to its movement ceases. On the withdrawal of the trocar the clear cerebrospinal fluid gushes out entirely free from blood.

"There is no necessity to tie the cannula in any way, since it is firmly fixed in the compact tissues in the back of the neck. A glass tube is now connected to the cannula by a short rubber connection and the cerebrospinal fluid is allowed to drip into a glass capsule. The fall of each drop is signalled electrically on the base line of the arterial pressure which is simultaneously taken."

Obtaining Cerebrospinal Fluid.—Tigerstedt, 3, 4, 133; Weed and Cushing, 1915, Amer. Jour. Physiol., 36, 77; *Chemic Examination*, Abderhalden, 5, 215.

Lumbar Puncture.—Tigerstedt, 3, 4, 8.

Artificial Hydrocephalus.—Frazier and Peet, 1914, Amer. Jour. Physiol., 35, 268.

EXERCISE X, A.—(OPTIONAL) ABSORPTION AND EXCRETION ON EFFECT (POTASSIUM CHLORID)

Anesthetize a dog with morphin and ether. Arrange for blood-pressure tracing. Ligature pylorus. Inject by stomach-tube KCl, 2 gm. per kg., diluted with water. Note that the blood-pressure does not change materially within an hour. Now ligature the renal vessels and repeat the KCl: the blood-pressure falls gradually.

QUESTIONS

- (a) Why is the potassium ineffective by stomach?
- (b) How can it be made effective? Why?

EXERCISE X, B.—(OPTIONAL) ABSORPTION INTO BLOOD AND LYMPH

Anesthetize an animal and place cannula into the ureters and thoracic duct. Inject methylene-blue solution into the peritoneal or pleural cavity: the color appears in the urine before the lymph (Starling and Tubby).

QUESTION

Does the absorption of the dye from serous cavities occur by the blood or lymph?

(D) IDIOSYNCRASY; ATROPIN; THYROID TEST

Introduction.—Idiosyncrasy is the term applied to an abnormal reaction to a drug. The abnormality is generally quantitative only; but it may appear qualitative by bringing into prominence some action of the drug

which is ordinarily so small as to escape observation. Most instances of idiosyncrasy may therefore be brought under the headings of exaggerated susceptibility or tolerance. These may be congenital or acquired. Some are readily explained by anatomic or physiologic peculiarities. Others are due to differences in the absorption, excretion, or destruction of the poison. Many phenomena of idiosyncrasy have not yet been satisfactorily explained. The continued administration of a drug often alters the susceptibility of the patient to its action; this may be diminished (*habituation*) or increased (*cumulative action*). Congenital idiosyncrasy may be individual or racial. The student will probably encounter some examples of individual idiosyncrasy in the course of his future work. The following experiments refer mainly to racial idiosyncrasy.

EXERCISE XI.—(GROUP IV) ATROPIN ON DOG AND RABBIT

(REPORTER I, A)

On a dog (A) and rabbit (B) observe the normal pulse, pupils, and respiration. Confirm also that the rabbit reacts to inhalation of ammonia by temporary arrest of the heart (trigeminal-vagus reflex).

Inject each animal, hypodermically, with Atropin, 5 mg. per kg. (0.5 c.c. of 1 per cent. per kg.). Repeat the observations from time to time. The effects are very much greater in the dog than in the rabbit (the heart rate is quickened by paralysis of the vagus endings; the respiration is first increased, then diminished. The general symptoms are first excitant, later depressant; the pupils are dilated through paralysis of the oculomotor endings). Let the rabbit inhale a little ammonia while feeling the heart-beat: the heart is not stopped, as it would be in normal animals.

It will have been noted that the pulse-rate is greatly quickened in the dog, but scarcely, if at all, in the rabbit. This is because in the dog the heart is normally kept slow by the tonic activity of the vagus center. This is cut out by atropin. These tonic impulses are very weak or absent in the rabbit, so that their abolition does not alter the heart-rate. The ammonia experiment shows that the atropin has paralyzed the vagus in the rabbit as well as in the dog.

The general resistance of rabbits is due, at least partly, to the more rapid destruction of atropin in their tissues.

QUESTIONS •

- (a) Describe the effects of atropin on general behavior; pulse; pupils; respiration.
- (b) Why does the effect on the pulse differ in dogs and rabbits?
- (c) How can it be shown that the vagus is paralyzed in rabbits?

EXERCISE XII.—(OPTIONAL) DIGITALIS ON TOAD AND FROG HEART

Apply some (10 per cent.) infusion of *digitalis* in 0.75 NaCl solution to the exposed heart of a pithed toad and frog, and notice that the effect on the frog is much greater. (Observe that the heart is slowed and the systole increased, peristaltic waves and arrhythmia become apparent, and the heart may be arrested in systolic standstill as a small white lump.)

The skin of the toad secretes a poison with an action analogous to digitalis. The tolerance of this animal is therefore somewhat analogous to habituation.

EXERCISE XIII, A.—(OPTIONAL) ACETONITRIL TEST FOR THYROID (HUNT'S METHOD)

The feeding of thyroid to mice greatly increased their resistance to acetonitril, presumably by diminishing the liberation of HCN. This serves as a qualitative and even quantitative test for thyroid substance.

White mice are fed for some weeks on a uniform diet (oats and water). The M. F. D. of acetonitril, hypodermically injected, is ascertained (beginning with 0.25 mg. per gm., fresh solution). This serves as a control. The thyroid preparation (say 1 mg. per day per mouse) is made into pills with cracker-dust and syrup, and these are added, for about ten days, to the diet of some mice of the same lot kept under the same conditions. At the end of this time the M. F. D. of acetonitril is determined on these mice (starting with 3, 6, 9, 12 times the normal dose).

EXERCISE XIII, B.—(OPTIONAL) TADPOLE TEST

Feeding of thyroid to tadpoles hastens their development, but checks growth (Guderatsch, 1912, Arch. Entwickl., 35, 457). Marine and Feiss, 1915 (Jour. Pharmacol., 7, 572), perform the test by feeding five tadpoles with 50 mg. of powdered thyroid every other day; on the alternating days the animals are fed with fresh sheep liver for two hours.

TECHNICAL REFERENCES

- Acetonitril Test*.—Hunt, 1909, Hyg. Lab. Bul. No. 47; Fuehner, 153.
Mice.—Keeping and breeding: Abderhalden, 3, 1268; Anesthesia, *ibid.*, 3, 1281; Injection, Fuehner, 148.
Thyroidectomy.—Abderhalden, 6, 560.
Thyroid Experiments.—Kobert, Intox., 1, 267.
Cretenism.—In young rats, by complete thyroidectomy, Basinger, 1916, Arch. Int. Med., 17, 260.

(E) EMETICS

Introduction.—These illustrate phases of racial idiosyncrasy, but the subject has also a direct practical importance.

Emetics are divided into two classes: Those which stimulate the vomiting center in the medulla directly (*central emetics*) and those which stimulate it reflexly (*local emetics*). The central emetics act at least equally well when they are injected hypodermically. Apomorphin is the principal example. Local emetics act by irritating the sensory endings in the pharynx or stomach. They are effective only if they are administered or excreted by this channel. All irritants belong to this class; but only those are practically useful which have only a slight toxicity, or which act so promptly that they are expelled before absorption can occur.

If a drug produces vomiting when injected into the circulation, and not when it is given by mouth, its action is surely central; and vice versa. If it causes emesis in either case, the relative quantity and the time required are taken into consideration: if it is more efficient by the circulation, its action is, at least mainly, central; and vice versa. The absolute distinction is made by ligating all the vessels of the stomach, exclusive of the nerves: a centrally acting emetic will now be effective only when injected into the circulation, a local emetic only when placed in the stomach.

Emesis, the act of vomiting, is preceded by nausea, and followed by depression. The relative duration of these stages is of great practical importance.

Observations to be Made.—The onset and duration and symptoms of nausea; onset and frequency of emesis; pulse and respiration of normal animal in nausea, just before, during, just after, and some time after vomiting. Note how soon the animal will drink water and eat meat again. Report the results. The animals should have been recently fed.

EXERCISE XIV.—(GROUP V) APOMORPHIN

(REPORTER II, A)

Inject Apomorphin hypodermically as follows, and observe effects:

Dog A—1 mg. (0.1 c.c. of 1 : 100) per kg.

Cat B—5 mg. (0.5 c.c. of 1 : 100) per kg.

Cat C—50 mg. (5 c.c. of 1 : 100) per kg.

Rabbit D—10 mg. (1 c.c. of 1 : 100) per kg.

(Optional) **Apomorphin as Hypnotic.**—This effect may be produced, rather uncertainly, by small doses (0.04 mg. per kg. for cats or dogs, hypodermically).

QUESTIONS.

(a) Describe the phenomena of apomorphin-vomiting as witnessed in Dog A and Cat C.

(b) Describe the phenomena of apomorphin-nausea as witnessed in Cat B.

(c) Describe the phenomena of apomorphin excitement as witnessed in Rabbit D.

(d) Why does the rabbit fail to vomit?

(e) Why is the cat less susceptible to apomorphin? Is it generally resistant to emetics?

(f) Is the action of apomorphin central or local? How could this be proved?

EXERCISE XV.—(OPTIONAL) LOCATION OF APOMORPHIN ACTION

(See Eggleston and Hatcher, 1912, Jour. Pharmacol., 3, 551.)

EXERCISE XVI.—(GROUP I, A, II, A, III, A) LOCALLY ACTING EMETICS

(REPORTER II, A)

Administer the following solutions to cats (or dogs, with double dose) by stomach-tube:

(Group I, A) Copper Sulphate, 25 c.c. of 1 per cent.

(Group II, A) Zinc Sulphate, 25 c.c. of 1 per cent.

(Group III, A) Tartar Emetic, 10 c.c. of $\frac{1}{4}$ per cent.

(Optional).—Ipecac (1 c.c. of fluidextract). Mustard (teaspoonful) in warm water. Ammonium Carbonate (10 c.c. of 5 per cent. solution). Senega (2 c.c. of fluidextract).

QUESTIONS

Describe the phenomena of nausea and vomiting; onset and duration; amount of depression.

(F) ANTEMETICS

Emesis may be treated either by depressing the vomiting center or (with the locally acting emetics) by protecting the stomach against local irritation.

EXERCISE XVII.—(GROUP I, B AND II, B) PARALYSIS OF VOMITING CENTER

(REPORTER II, A)

Experiment 1. (Group I, B) Morphin and Apomorphin.—Inject into dog 10 mg. per kg. ($\frac{1}{4}$ c.c. per kg. of 4 per cent.) of morphin, subcutaneously.

This will cause vomiting, probably by an action similar to apomorphin (which is a derivative of morphin). After half an hour inject apomorphin 1 mg. (0.1 c.c. of 1 per cent.) per kg. hypodermically. This will be ineffective, as the morphin stimulation of the vomiting center is followed by a profound depression. All other emetics will be similarly ineffective. This is utilized in experimental technic, when it is essential to have an irritant drug retained in the stomach.

Experiment 2. (Group II, B) Morphin and Zinc Sulphate.—Proceed as in Experiment 1 (dog or cat), but use 50 c.c. of 1 per cent. zinc sulphate (25 c.c. for cat) by stomach-tube in place of the apomorphin.

QUESTIONS

- (a) How does morphin affect emesis?
- (b) Does it act against both central and local emetics?
- (c) Would it be available as a therapeutic measure?

EXERCISE XVIII.—(GROUP III, B) BISMUTH AND ZINC SULPHATE

(REPORTER II, A)

Administer to a cat, by stomach-tube, 1 gm. of bismuth subcarbonate, suspended in 50 c.c. of mucilage of acacia. In ten minutes follow this by 25 c.c. of 1 per cent. zinc sulphate, also by stomach-tube: vomiting will be delayed or prevented.

QUESTIONS

- (a) Would bismuth be effective against both classes of emetics (central and local)?
- (b) Could it be used clinically? Against which conditions?

TECHNICAL REFERENCES ON DIGESTIVE TRACT

- Operations.*—London in Abderhalden, 3, 76.
Digestion Experiments on Animals.—Zunz, *ibid.*, 3, 122.
Collection of Digestive Secretions.—*Ibid.*, 3, 189.
Products, Collection, and Analysis.—*Ibid.*, 6, 458.
Examination of Stomach Contents.—Abderhalden, 8, 44.
Indicators of Gastric Acidity.—Fowler, Bergheim, and Hawk, 1915, Soc. Exp. Biol. Med., 13, 58.
Aseptic Technic.—Tigerstedt, 1.1, 55; 3.4, 16; Hoskins and Wheelan, 1914, Amer. Jour. Physiol., 34, 81. Iodin as skin disinfectant, Mayers, 1911, Soc. Exp. Biol. Med., 8, 53.
Digestive Fistula.—Permanent: *Ibid.*, 6, 564; Thiry-Vella, *ibid.*, 6, 466.
Eck's Fistula.—Abderhalden, 6, 528; Peet, Ann. Surg., Nov., 1914.
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Pancreatic Juice.—Abderhalden, 6, 488.
Pancreas Experiments.—Kobert, Intox., 1, 265.
Bile Secretion.—*Ibid.*, 1, 262; Exclusion from Intestine, Pearce and Eisenbrey, Amer. Jour. Physiol., 32, 417.
Visceration of Animals.—Barcroft and Brodie, 1904, Jour. Physiol., 32, 19.
Splenectomy.—Abderhalden, 6, 561.

CHAPTER XXXIX

METABOLISM; DEPRESSANTS; IRRITANTS. (A) TEMPERATURE; (B) GLYCOSURIA; (C) METABOLISM; (D) CENTRAL DEPRESSANTS AND TREATMENT OF DEPRESSANT POISONING; (E) GASTRO-ENTERITIS; (F) NEPHRITIS; (G) REFLEX EFFECTS OF IRRITANTS. ARSENIC ON BLOOD-PRESSURE

(A) EFFECTS ON TEMPERATURE

Introduction.—The temperature of an animal is determined by the relation of heat dissipation and heat production. The heat-regulating mechanism of warm-blooded animals is able to keep the temperature of the body constant, notwithstanding all ordinary variations of external and internal conditions. The temperature can therefore be altered only by very violent changes or, more commonly, by disturbing the regulating mechanism. Several centers are concerned in the latter. Successive stimulation or section of the paths is necessary to distinguish which of these is concerned in a given phenomenon. These experiments are rather complicated.

By the use of the calorimeter and by the study of metabolism it is easy to determine whether the change of temperature is due to altered heat production or heat loss. The plethysmograph will show whether changes in heat loss are due to an action on cutaneous vessels. The evaporation of sweat may be excluded by atropin, which paralyzes the sweat-glands.

The *drugs which increase temperature* act generally on heat production by increasing muscular movement. Cocain acts on the centers of the caudate nuclei. The hypodermic injection of irritants, even of water, and especially of albumose, produces some hyperpyrexia in rabbits. Bacterial toxins are the most efficient pyretics.

The *drugs which lower temperature* may do so by producing a general depression of the central nervous system—a shock or collapse action. Alcohol, chloral, morphin, etc., belong to this class. These lower the temperature even in previously healthy individuals.

The *typical antipyretics*, on the other hand, lower the temperature only when it is abnormally high, *i. e.*, in fever; and then only to normal. The coal-tar antipyretics (acetanilid, antipyrin, etc.) act centrally, and increase the heat dissipation by dilating the cutaneous capillaries. Quinin diminishes the heat production by a direct action on the muscular metabolism.

Observations Required.—Rectal temperature (every half-hour). The observations should be made before giving the drugs, and the animal should be kept and observed under perfectly uniform conditions, to exclude accidental variations. (The effects are usually seen in two to three hours.)

The thermometer must be oiled and inserted always to the same depth (2 or 3 inches). With small animals the bulb should be warmed in the hand. Rabbits have a more responsive temperature than cats, but these may be substituted, using the same doses per kilogram. Plot curves of the temperature.

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General Discussion.—Kobert, Intox., 1, 200, 280.

Heat Puncture.—Tigerstedt, 3, 4, 86; Aaronsohn and Sachs, 1885, Arch. ges. Physiol., 37, 232; Gottlieb, Arch. exp. Path., 26, 422; Jacoby, Exp. Ther., 151.

Beta-Tetrahydronaphthylamin.—Jonescu, Arch. exp. Path., 60; Elliott, 1914, Quart. Jour. Med., 7, 120, claims that rabbits show *gastric ulcers* a few hours after hypodermic injection.

Calorimetry.—Abderhalden, 3, 1158; 7, 658; Tigerstedt, 1, 3, 150; Lusk, 1915, Arch. Int. Med., 15, 793; *Small Animals*, Langworthy and Milner, 1916, Jour. Agr. Res., 6, 703.

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Heating of Carotid Blood.—Stewart, 297.

Experimental Infections.—*Rheumatic Arthritis*, Klotz, 1914, Cleve. Med. Jour., 13, 210; Rothschild and Thalheimer, 1914, Jour. Exp. Med., 19, No. 5; *Synovitis*, Andrei, 1913, Zentr. Bioch. Bioph., 16, 341; *Pneumonia*, Lamar and Meltzer, 1910, Soc. Exp. Biol. Med., 7, 102; Wolfenstein and Meltzer, 1912, Jour. Exp. Med., 16; Kline and Winternitz, 1915, ibid., 21, 304; Kline and Meltzer, 1915, Soc. Exp. Biol. Med., 13, 29 (unorganized substances); Sisson and Walker, 1915, Jour. Exp. Med., 22, 747 (Friedlander type); *Syphilis*, Rabbits, Jacoby, Exp. Ther., 99; *Trypanosomes*, Abderhalden, 5, 1371.

Scurvy, Experimental.—L. Jackson and Moore, 1916.

EXERCISE I.—(GROUP I, A) CHLORAL (FALL OF TEMPERATURE BY COLLAPSE)

(REPORTER III, A)

Administer by stomach-tube to cat chloral, 0.5 gm. (20 c.c. of 2.5 per cent.) per kg.: there is a fall of temperature, general depression, and partial or complete coma. The respiration is slower and more shallow. (Depression of medullary centers.)

EXERCISE II.—(GROUP II, A) MORPHIN (FALL OF TEMPERATURE BY DIMINUTION OF METABOLISM, AND PERHAPS BY A SPECIFIC EFFECT ON TEMPERATURE CENTERS)

(REPORTER III, A)

Inject hypodermically into rabbit 0.01 gm. per kg. ($\frac{1}{4}$ c.c. per Kg. of 4 per cent. solution). The effects resemble those of chloral, but are not so severe. (Test urine for sugar.) A respiratory tracing may be taken if the animal shows Cheyne-Stokes respiration.

EXERCISE III.—(GROUP III, A) SANTONIN (FALL, THEN RISE)

(REPORTER III, A)

Inject into the stomach of a rabbit 0.5 gm. per kg. of Santoninate of Sodium (10 c.c. per kg. of 5 per cent.): there is at first a fall of temperature due to the increased heat loss. Convulsions set in, and when these are violent the temperature may rise on account of the increased muscular activity. When the convulsions give place to paralysis there is a second more profound fall of temperature. (Santonin illustrates typically the effect of all convulsant poisons on temperature.)

EXERCISE IV.—(GROUP IV, A) COCAIN (RISE OF TEMPERATURE THROUGH STIMULATION OF THE CAUDATE NUCLEUS)

(REPORTER III, A)

Inject hypodermically into rabbit, cat, or dog cocaine, 25 mg. (0.5 c.c. of 5 per cent.) per kg.: rise of temperature of 1° to 2° C. The animal may show great excitement and even violent convulsions.

EXERCISE V.—(GROUP IV, B) BETA-TETRAHYDRONAPHTHYLAMIN

(REPORTER III, A)

Inject hypodermically into rabbit 25 to 50 mg. ($\frac{1}{2}$ to 1 c.c. of 5 per cent.) per kg.: rise by strong cutaneous vasoconstriction and increased movements.

EXERCISE VI.—ALBUMOSE FEVER AND ANTIPYRETICS

(REPORTER III, A)

Use rabbits or, if necessary, cats.

Experiment 1. (Group V, A) Albumose (Rise of Temperature).—Inject hypodermically 1 gm. per kg. (5 c.c. per kg. of 20 per cent.): rise.

Experiment 2. (Group V, A) Antipyrin (Little Effect on Normal Animals).—Give 0.1 gm. per kg. (10 c.c. per kg. of 1 per cent.) hypodermically. There is little, if any, effect.

Experiment 3. (Group V, B) Antipyrin in Fever (Regulation of Temperature).—Give albumose, as in Experiment 1, and follow this in two to four hours by antipyrin (as in Experiment 2). The temperature soon returns to normal, while that of Experiment 1 remains high.

QUESTIONS

- (a) State which drugs raise, and which lower, temperature.
- (b) Are the antipyretics equally efficient in the absence of fever?

EXERCISE VII.—(OPTIONAL) ASSAY OF ANTIPYRETIC EFFICIENCY

(See Kiliani, 1910, Arch. Internat. Pharmacodyn., 20, 333; Fuchner, 157.)

(B) GLYCOSURIA

Introduction.—The presence of sugar in the urine may be due to several different causes. These are discussed in text-books of physiology.

The presence of reducing substance in the urine, after the administration of drugs, is often due to glycuronic acid, which is generally excreted in paired combination with the drug. These urines reduce Fehling's solution, but do not give the fermentation test.

The following are examples of drugs that cause the appearance of glycuronic acid: Copaiba, Chloral, Menthol, Thymol, many volatile oils, Carbon-monoxid, Chloroform, Formates, free Oxalic Acid, Benzaldehyd, Morphin.

True glycosuria (in which the urine also gives the fermentation test) is caused by: Phlorhizin, Epinephrin, Uranium, Curare, Cyanids, Atropin, Amyl Nitrite, Chromates and Bichromates, Mercury, Morphin, Cantharidin, extensive salt injections, etc.

Many of these act by producing asphyxia. Phlorhizin acts directly on the kidney cells.

TECHNICAL NOTES

Catheterization requires considerable practice in dogs and in female rabbits; it is easy in male rabbits. A No. 5 bone-tipped gum male catheter is used. The urine of rabbits may be collected by *expression*: The animal is grasped firmly in the left hand, so as to push the abdominal organs toward the pelvis, when moderate pressure with the right hand, over the bladder, usually accomplishes the desired result. The urine and feces may also be collected by placing the animals in suitable *Metabolism cages*.

TECHNICAL REFERENCES

Catheterization of Animals.—Stewart, 690; Abderhalden, 3, 1045; *Separation*, Tschermichowski, 1909, Bioch. Cbl., 8, 932.

Glycosuria.—Stewart, 690; Abderhalden, 5, 1199.

Sugar Estimation.—Abderhalden, 2, 167 (in blood, *ibid.*, 5, 172); Shaffer, 1914, *Jour. Biol. Chem.*, 19, 285; Lewis and Benedict, 1915, *ibid.*, 20, 61; Macleod, "Diabetes," 6, 11, 16, 26; *Clinical*, Kleiner, 1914, *Jour. Amer. Med. Assoc.*, 62, 1307; *Comparison of Methods*, Fitz, 1914, *Arch. Int. Med.*, 14, 133; Morris, 1916, *Jour. Lab. Clin. Med.*, 1, 252; *Normal Human Urine* (qualitative), Folin, 1915, *Jour. Biol. Chem.*, 22, 327; *Determination of Small Amounts in Urine*, V. C. Myers, 1916, *Proc. Soc. Exp. Biol. Med.*

Glycuronic Acid.—Abderhalden, 2, 101, 139; 3, 949, 969; in blood, *ibid.*, 5, 177; paired, *ibid.*, 6, 258.

Acetonuria, Experimental.—(Phlorizin with fasting), Jacoby, *Exp. Ther.*, 143.

Diabetes Insipidus, Experimental.—S. A. Matthews, 1915, *Arch. Int. Med.*, 15, 451.

EXERCISE VIII.—(GROUP II, A) MORPHIN (ASPHYXIAL CONVERSION OF GLYCOGEN INTO GLUCOSE) (See EXERCISE II)

EXERCISE IX.—(OPTIONAL) PHLORHIZIN

(Renal action.) Inject hypodermically into a rabbit $\frac{1}{4}$ gm. of phlorhizin dissolved in 5 c.c. of warm water. Keep the animal in a cage arranged for the collection of urine. If none has been passed in an hour, withdraw by a catheter, and demonstrate the presence of sugar by Fehling's or Trommer's tests.

EXERCISE X.—(OPTIONAL) EPINEPHRIN

Inject subcutaneously into a rabbit 1 to 2 c.c. of 1 : 1000 epinephrin; in two hours collect the urine and test for sugar.

(C) METABOLISM

Introduction.—Drugs may alter metabolism directly by acting on the tissues or on certain nervous centers; or indirectly by influencing digestion, absorption, or excretion; or by making the animal quiet or restless, etc.

The experimental investigation of nitrogen or carbon metabolism entails extensive preparation and surveillance of the animals and time-consuming analytic methods. The following experiments are, therefore, optional.

EXERCISE XI.—(DEMONSTRATION) ACID INTOXICATION

(REPORTER V, A)

Administer to a rabbit, by stomach-tube, 100 c.c. of 1 per cent. HCl per kg.: unsteady motions, slowed heart and respiration, stupor, coma, convulsions, air-hunger, but no cyanosis. Death may occur in twelve to forty-five minutes. Just before death insert a cannula into the jugular vein, toward the heart, and inject slowly a 0.5 per cent. solution of sodium carbonate: recovery.

EXERCISE XII.—(OPTIONAL) EXPERIMENTS ON NITROGEN METABOLISM

Dogs or rabbits may be used. Arrange for the regular collection of urine. The animals may be reduced to nitrogen equilibrium and then kept on a uniform diet; or they may be starved until the nitrogen is practically constant. The urine may be examined for total nitrogen and for urea. The following drugs may be tried:

Quinin: 0.05 per kg.

Antipyrin: 0.2 gm. per kg.

Water: large quantity.

The following drugs are important: Quinin diminishes nitrogen metabolism; the coal-tar antipyretics also, but only in fever. Morphin diminishes carbon metabolism. Phosphorus in toxic doses increases nitrogen metabolism, but diminishes urea. Acids increase ammonia excretion at the expense of urea; alkalis the reverse. Salts and water increase nitrogen excretion.

TECHNICAL REFERENCES

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- Respiration Chamber.**—*Man*, Langworthy and Milner, 1915, Jour. Agr. Res., 5, 299. *Small animals*, Kolls and Loewenhardt, 1915, Amer. Jour. Physiol., 39, 67; Benedict, 1915 (CO₂ and oxygen), Jour. Biol. Chem., 20, 301.
- Animals, Apparatus.**—Cages, Feed, etc., Abderhalden, 5, 1035; Tigerstedt, 1.3, 1.
- Tread-mill**, Abderhalden, 3, 1050.
- Dogs (and Feed).**—Abderhalden, 3, 1041; Pratt, 1909, Feeding, Amer. Jour. Physiol., 24, 269.
- Ruminants.**—Abderhalden, 3, 1054; collection excreta, *ibid.*, 6, 453.
- Mice, Guinea-pigs, etc.**—Feeding, Abderhalden, 3, 1269.
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- Meat, Protein Content**, Bedford and Jackson, 1916, Proc. Soc. Exp. Biol. Med., 13, 83.
- Feces.**—*Delimitation*, Abderhalden, 3, 999; 5, 333. (Charcoal, 0.2 gm., or carmin, 0.3 gm. in capsule before breakfast appears in stool of next morning.) *Examination of human*, Abderhalden, 5, 331. *Examination of herbivorous*, *ibid.*, 3, 263. *Preservation*, Howe, Rutherford, and Hawk, 1910, Jour. Amer. Chem. Soc., 32, 1683. *Fat*, Abderhalden, 5, 363; Saxon, 1914, Jour. Biol. Chem., 17, No. 2. *Bacteria*, Abderhalden, 5, 359. *Ash*, *ibid.*, 5, 331.
- Urine.**—*Collection*, human, Abderhalden, 3, 999; 5, 281; pan for quantitative collection of female urine, Folin and Denis, 1915, Arch. Int. Med., 16, 195. *Preservation*, *ibid.*, 5, 283; Gill and Grindley, 1909 (Thymol and cold), Jour. Amer. Chem. Soc., 31, 695. *General Urine Analysis*, Abderhalden, 3, 765; 5, 281.
- Nitrogen.**—Folin, 1915, Jour. Biol. Chem., 21, 195; Bock and Benedict, 1915, *ibid.*, 20 No. 1; Gradwohl, 1916, Jour. Amer. Med. Assoc., 67, 809.
- Urease**, Dunning, 1916, Amer. Jour. Phar., 5, 809; *for Urea in Urine*, Fiske, 1916, Jour. Biol. Chem., 23, 455; *in blood*, Marshall, 1913, *ibid.*, 14, 283; 15, 487.
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- Purin Bases.**—Graves and Kober, 1915 (nephelometric), Proc. Amer. Soc. Biol. Chem. 3, 17.
- Creatin and Creatinin.**—Morris, 1915, *ibid.*, 3, 15; Janney and Blatherwick, 1915, Jour. Biol. Chem., 21, 567 (in muscle and organs).
- Phenols in Urine and Feces.**—Folin and Denis, 1916, Jour. Biol. Chem., 26, 507.
- Bence-Jones Proteinuria.**—Folin and Denis, 1914, Jour. Biol. Chem., 18, 277.
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- Carbon Dioxid, Tension in Alveolar Air.**—Marriott, 1916, Jour. Amer. Med. Assoc., 66, 1594; *Combining Power of Plasma*, Van Slyke, Stillman, and Cullen, 1915, Soc. Exp. Biol. Med., 13, 39.
- Birds.**—Abderhalden, 3, 1058; *Anesthesia, operations, urine secretion*, Sharpe, 1912, Amer. Jour. Physiol., 31, 75.
- Fish.**—*Urine Collection*, Denis, 1912, Jour. Biol. Chem., 13, 225. *General Experiments*, Fuehner, 52; *Operative Technic*, Abderhalden, 3, 1103; *Isolated heart*, Beresin, 1913, Arch. ges. Physiol., 150, 549.
- Marine Animals.**—Abderhalden, 3, 1064.
- Micro-organisms.**—Abderhalden, 5, 1158.
- Perfusion.**—*Ibid.*, 5, 1245.
- Surviving Organs.**—Baglioni in Abderhalden, 3, 358.

(D) CENTRAL DEPRESSANTS

Introduction.—The effects of central depressants, as seen in mammals, are not as sharply localized as in frogs. The symptoms usually begin with stupidity and drowsiness, with or without excitement; ataxia, sleep, and coma. The respiration is usually slowed, more than corresponds to the muscular quiet. The reflexes are usually diminished, but with morphin they may be increased. The temperature tends to fall. The details of these actions determines their practical availability as analgesics, hypnotics, or anesthetics.

Depression of the brain interferes in the first place with the higher psychic processes; this passes into sleep, and finally into anesthesia. Depression of the spinal cord leads to loss of reflex excitability; depression of the medulla, to fall of blood-pressure, quickening of the pulse, slowing of respiration, and fall of temperature. The location of the action of the depressants is therefore indicated by the symptoms.

The readiness with which the successive stages may be produced and their duration varies with each drug, and determines its uses in therapeutics.

Those which act mainly on the higher centers are used for the relief of pain (analgesics) or for producing sleep (hypnotics): Morphine, Cannabis, Alcohol, Chloral.

Those which act profoundly on the brain and spinal cord are employed for operative anesthesia (general anesthetics): Ether, Chloroform, Ethyl Chlorid, etc.

Paralysis of the medulla (Chloral, Chloroform) is only utilized in experimental technic; but it is important as a source of danger in anesthesia. It is treated mainly by artificial respiration.

It will be remembered that most central stimulants also produce some depression; similarly, the depressants often cause some stimulation. Morphine may produce excitement in certain individuals; it may also stimulate the vomiting and defecating and motor centers. It always increases the reflex excitability of the spinal cord, and may even cause typical strychnin spasms in the lower animals.

Alcohol and the general anesthetics produce a preliminary stimulation. This, however, is not due to a direct stimulant action, but to inhibition of restraining centers and to reflex stimulation.

Observations.—In observing the effects, attention should be directed especially to the general behavior of the animal (excitement, drowsiness, ataxia, sleep, coma, etc.); to the respiration, pulse, and temperature; the reflexes (patellar, ear, etc.); the pain reaction (sudden and gradual pressure on foot), etc. These should be observed before and at intervals after the administration. Care must be taken that the animal is not excited when the normal observations are taken. If the respiration becomes irregular, endeavor to obtain tracings (with a lever attached by a bulldog clamp to the hair of the chest or abdomen).

TECHNICAL REFERENCES

Central Depressants.—Kobert, *Intox.*, 1, 223.

Psychologic Tests.—Tigerstedt, 3-5; *Mental tests*, Dana, 1913, *Med. Rec.*, Jan. 4; Binet Scale, *Pop. Sci. Mo.*, Jan., 1914.

EXERCISE XIII.—MORPHINE

(REPORTER IV, A)

Experiment 1. (Group II, A) Dog.—Inject hypodermically 10 mg. ($\frac{1}{4}$ c.c. of 4 per cent.) per kg. and carefully observe the effects. The animal will probably vomit and pass feces and sometimes urine (stimulation of medullary and spinal centers). The respiration may be temporarily quickened, but will soon become slowed and more shallow (stimulation and depression of the respiratory center). A tracing may be taken. The pulse-rate will decrease (stimulation of vagus center). The temperature falls (general lowering of metabolism). The pupils are variable (central action). The animal becomes more quiet; does not move spontaneously, and the movements are shivering. The hind legs are especially affected, and may be dragged when the animal walks. The dog does not usually fall asleep, but pain is felt less acutely. The reflexes, however, are not diminished.

The effect is, on the whole, a central depression; the action differs from that on man mainly by the absence of sleep, and by the presence of the diarrhea, by the variability of the pupils, and by the more pronounced motor disturbances.

Experiment 2. (Group II, B) Cat.—Inject hypodermically 20 mg. ($\frac{1}{2}$ c.c. of 4 per cent.) per kg. The effect may be excitant, the animal running about; the pupils dilate; however, analgesia is present.

Experiment 3. (Group II, A) Rabbit.—See Exercise II.

Questions.—(a) Describe the effects of morphin on the three animals.

(b) Which is most and which least susceptible to the narcotic action (considering the dosage)?

(c) What are the most conspicuous qualitative differences in the actions?

Experiment 4. (Demonstration) Mouse Test for Morphin.—The hypodermic injection of morphin into white mice is followed in two to twenty minutes by a peculiar position of the tail, which is carried in a rigid, usually **S** curve over the back. This is maintained for one or two hours.

The reaction is characteristic for morphin (above 0.01 mg. for mouse of 15 to 20 gm.); it is also given by some of the other opium alkaloids and apomorphin.

Inject under back of white mouse morphin 0.5 mg. (0.5 c.c. of 1 : 1000) and observe results.

Technical References.—Straub, 1911, Deut. med. Woch., 37, 1462; Fuehner, Nachweiss, 150.

Experiment 5. (Optional) Synergism of Opium Alkaloids. (See W. Straub, 1912, Bioch. Zs., 41, 4191.)—Inject hypodermically into white mice the following drugs, and note whether they survive or die. The dosage refers to mice of 15 to 20 gm.:

(1) Morphin, 12 mg.

(2) Morphin, 18 mg.

(3) Narcotin, 10 mg.

(4) Narcotin, 2 mg., with morphin, 2 mg.

(5) Narcotin, 4 mg., with morphin, 4 mg.

(1), (3), and (4) should survive; (2) and (5) should die.

Questions.—(a) What effect has narcotin on the toxicity of morphin? (Compare 1 and 2 with 3 and 4.)

(b) Is this a simple addition of the toxicity of the two drugs?

(c) What is this action called?

Experiment 6. (Optional) Papaverin.—Inject cat, hypodermically, with 100 mg. per kg.: narcosis.

Experiment 7. (Optional) Synergism of Morphin, Scopolamin, and Atropin, Cat.—Inject three cats hypodermically with morphin, each 20 mg. ($\frac{1}{2}$ c.c. of 4 per cent.) per kg. Use Cat A as control. Into Cat B inject Scopolamin, 0.5 mg. per kg. ($\frac{1}{2}$ c.c. of 1 : 1000); into Cat C inject Atropin, 1 mg. per kg. (1 c.c. of 1 : 1000). Inject Cat D with Scopolamin and Cat E with Atropin, using the same doses, both without Morphin. Compare the results.

EXERCISE XIV.—CANNABIS

(REPORTER IV, A)

The administration of cannabis to dogs usually produces vomiting and some excitement. In one or two hours this is followed by muscular incoördination (ataxia), and, finally, by lassitude, depression, and sleep. The individual susceptibility varies. Small, short-haired dogs (fox terriers) are most suitable. The effects do not occur on hypodermic administration. The activity is due to resinous constituents.

Experiment 1. (Group III, B) Effects.—Administer a capsule containing extract of Cannabis Indica 0.05 gm. per kg. This is done by drawing out the tongue and placing the capsule back as far as possible. On releasing the tongue the capsule is usually swallowed easily. If not, the mouth is held shut and the animal slapped on the throat. Observe the effects of the cannabis.

Questions.—(a) Describe the effects of the cannabis.

(b) Why is it inactive hypodermically?

Experiment 2. (Optional) Bio-assay of Cannabis.—This is performed similarly to Experiment 1. Details, U. S. P. IX. The standard dose for producing muscular incoordination in dogs is per kg.: fluidextract, 0.03 c.c.; extract, 0.004 gm.

Technical References.—U. S. P. IX; Pittenger, 98; Jour. Amer. Pharm. Assoc. (Committee), 1, 1305, 1912; Houghton, 1911, Amer. Pharm. Assoc. Bul., 6, 176.

EXERCISE XV.—(DEMONSTRATION) MAGNESIUM AND CALCIUM

(REPORTER IV, A)

Magnesium produces a depressant action, with sensory and motor paralysis, both central and peripheral. Calcium is also depressant, but nevertheless it antagonizes the magnesium effects, so that the animal recovers immediately.

A rabbit has received intramuscularly magnesium sulphate (crystals), 1.75 gm. (7 c.c. of 25 per cent.) per kg. When paralysis is complete, 6 to 8 c.c. of 3 per cent. calcium chlorid is injected slowly into the jugular vein: immediate recovery.¹

QUESTIONS

- (a) Describe the effects of magnesium.
- (b) Could this be utilized clinically for anesthesia?
- (c) Describe the effects of calcium on the magnesium rabbit.
- (d) Explain the antagonism.

TECHNICAL REFERENCE

Meltzer and Auer, 1907, Soc. Exp. Biol. Med., 5, 33.

EXERCISE XVI.—(GROUPS III AND IV) ALCOHOL AND TREATMENT OF ALCOHOL POISONING

(REPORTER IV, A)

Record respiration, temperature, and general symptoms (Pilcher, 1912, Jour. Pharmacol., 3, 267). Cats are used.

Experiment 1. (Group III, A) Alcohol Control.—Inject into cat by stomach-tube Alcohol 4 c.c. (16 c.c. of 25 per cent.) per kg. Observe symptoms and course for control.

Experiment 2. (Group III, B) Alcohol and Emesis.—Inject Alcohol as in Experiment 1. When symptoms are fully developed, or in about one-half hour, administer Zinc Sulphate, 25 c.c. of 1 per cent., by stomach-tube. Compare course with Experiment 1.

(If one of the cats should have vomited spontaneously, it will not be necessary to administer the emetic.)

Experiment 3. (Group IV, A) Alcohol-caffeine Antagonism.—Inject Alcohol as in Experiment 1. When symptoms have fully developed, or in about one-half hour, inject, hypodermically, Caffeine, 20 mg. (2 c.c. of 1 per cent.) per kg. Observe immediate effect and compare subsequent course with Experiment 1.

Experiment 4. (Group IV, B) Alcohol-caffeine Synergism.—Inject Alcohol as in Experiment 1. Follow this at once with a hypodermic injection of Caffeine, 50 mg. (5 c.c. of 1 per cent.) per kg. Compare course with Experiment 1.

¹ This rabbit may then be used for Exercise II.

QUESTIONS

- (a) Describe the phenomena of alcohol poisoning.
- (b) Name two methods of treatment and compare their efficiency as to immediate and ultimate improvement.
- (c) Does the antidotal efficiency of caffeine increase with the dose? Explain.

EXERCISE XVII.—(GROUP I) CHLORAL POISONING AND TREATMENT.

(REPORTER IV, A)

Chloral is a typical depressant. Cats are used. The effects increase with the dosage as follows (the dosage refers to gm. per kg., administered as 2.5 per cent. solution by stomach-tube to cats):

0.09 to 0.15: natural sleep.

0.18 to 0.25: light coma; recovery over night.

0.3 and higher: deep coma; recovery in one to four days.

0.35 to 0.50 (mean, 0.44): fatal.

Unless vomiting occurs (which is not infrequent), 0.5 gm. per kg. may be accepted as surely fatal.

Observe drowsiness; equilibrium; pain; reflexes; respiration; pupils; temperature.

Technical References.—Sollmann and Hatcher, 1908, Jour. Amer. Med. Assoc., 51, 487.

Experiment 1. (Group I, A) Symptoms of Chloral Poisoning.—See Exercise I. Administer by stomach-tube a fatal dose of chloral, 0.5 gm. (20 c.c. of 2.5 per cent.) per kg.

Experiment 2. (Group I, B) Chloral and Heat.—Proceed as in Experiment 1, but keep the animal warm. Compare the results.

Experiment 3. (Group I, A) Chloral and Caffein.—Inject Chloral as in Experiment 1. Fifteen minutes later give Caffein, 10 mg. (1 c.c. of 1 per cent.) per kg., hypodermically. Compare immediate and ultimate results.

Experiment 4. (Group I, B) Chloral and Strychnin.—Inject Chloral as in Experiment 1. After fifteen minutes begin treatment with Strychnin: administer 0.1 mg. (0.1 c.c. of 1 : 1000) per kg., hypodermically, and repeat every half-hour unless the animal becomes spasmodic. Compare immediate and ultimate results.

Experiment 5. (Optional) Chloral and Antidotes in Rabbits.—Administer to rabbits, by stomach-tube, Chloral, 0.5 gm. per kg. When light narcosis has set in, try the following drugs, by vein (doses are per kg.): immediate revival with Cocain, 5 mg. (may become convulsive); Caffein, 20 to 40 mg.; beta-tetra-hydronaphthylamin, 10 to 20 mg.

No revival: Phenol (but twitchings); Epinephrin or Pituitary (Y. Airila, 1913, Arch. Int. Pharmacod., 23, 453).

QUESTIONS

- (a) Describe the effects of chloral.
- (b) How may these be treated?
- (c) How do these methods compare in efficiency?
- (d) Are all the symptoms relieved to the same degree?
- (e) Suggest other methods of treatment.

(E) GASTRO-ENTERITIS

Introduction.—The most important phenomena of poisoning by irritants are caused by gastro-enteritis. The principal symptoms consist in very severe abdominal pain; profuse vomiting and diarrhea; and reflex collapse.

If the irritant is also corrosive, the discharges are bloody or otherwise discolored. The stools are generally very watery.

Observations Required.—Keep in cage and collect urine. During life note the vomiting, watery diarrhea, and general depression. At autopsy note the congestion of the abdominal organs, particularly the mucosa of the alimentary canal. Observe the character of the contents, and look for corrosions. Corrosions are most pronounced in the case of the mercury; they are absent with colchicum. The latter causes intense congestion in ridges. Observe that the arsenic produces its effects, even when it is given hypodermically (note particularly the fluid contents). The animal usually lives several hours or longer. The urine of mercury and arsenic will generally contain albumin and casts. The mercury and veratrin animals may recover, but will show erosion of the stomach on autopsy.

As these experiments would be painful, they are to be performed on the morphinized animals of Exercise XIII.

EXERCISE XVIII.—PHENOMENA OF GASTRO-ENTERITIS

(REPORTER V, A)

Experiment 1. (Group II, A) Colchicum.—Administer by stomach-tube to morphinized dog (or cat) Fluidextract of Colchicum 0.5 c.c. per kg.: no symptoms for several hours; but on the next day the animal will be found dead, with evidence of bloody diarrhea and hemorrhagic congestion of intestines. Autopsy.

The alkaloid of colchicum is practically inactive, but is converted in the tissues of mammals into oxydicolchin, which is the toxic agent. This explains the long interval between administration and symptoms. The drug is not at all corrosive. It has been suggested that it does not irritate directly, but that it merely exaggerates the normal irritability of the intestine.

Experiment 2. (Group II, B) Mercuric Chlorid.—Inject by stomach-tube into morphinized cat Mercuric Chlorid 5 mg. (5 c.c. of 1 : 1000) per kg.

Notice the white (cooked) appearance and hardness of the gastric mucosa at the autopsy.

Experiment 3. (Group II, A) Arsenic.—Inject hypodermically into morphinized rabbit Sodium Arsenate, 50 mg. (1 c.c. of 5 per cent.) per kg.

The symptoms and lesions of arsenic poisoning bear the closest resemblance to those of local inflammation of the alimentary tract. It can be shown, however, that the direct irritant or corrosive action of the poison is entirely inadequate to produce this inflammation, especially when the poison is given hypodermically or intravenously. Its action is really due to direct paralysis of the capillaries, with increased permeability. This is also the main phenomenon of inflammation. The lesions are therefore identical. A characteristic clinical feature of acute arsenic poisoning consists in the "rice-water" stools, which consist of a profuse watery exudate with shreds of desquamated mucosa.

QUESTIONS

Describe the symptoms and lesions of poisoning by colchicum, mercuric chlorid, and arsenic.

Experiment 4. (Optional) Veratrin.—1 c.c. of 1 per cent. by stomach, rabbit. Veratrin is one of the very few alkaloids which are directly corrosive.

TECHNICAL REFERENCES

Experimental Hepatic Cirrhosis.—Methods are described by: Pearce, 1906, *Jour. Exp. Med.*, January; Opie, 1910, *Trans. Assoc. Amer. Physicians*, 25; 1912, *ibid.*, 117; Grover, 1913, *Jour. Amer. Med. Assoc.*, 61, 458; Lissauer, 1914, *Arch. Path. (Virch.)*, 217, 56.

Spontaneous Hepatic Cirrhosis of Rabbits.—Grover, 1915, *Jour. Amer. Med. Assoc.*, 64, 1487.

Fatty Degeneration of Liver.—Phosphorus, Abderhalden, 5, 1232.

EXERCISE XIX.—(OPTIONAL) MORPHIN ON COLOCYNTH DIARRHEA

Administer to cat, by stomach-tube, 10 c.c. of a 10 per cent. infusion of Colocynth. After two hours decerebrate and expose intestines. They should be in violent peristalsis. Inject hypodermically Morphin, 20 mg.: the peristalsis should be promptly arrested (Padtberg, 1911, *Arch. ges. Physiol.*, 139, 318; Takahashi, 1915, *ibid.*, 159, 327).

(F) NEPHRITIS

Introduction.—The action of irritants is proportional to their concentration. This is greatest where they enter and leave the body—in the alimentary canal and in the kidneys. During their passage through the body they are generally diluted to such a degree that the irritation of other tissues is seen only when they are administered continuously. It may then lead to increased formation of fibrous tissue (arteriosclerosis and cirrhosis). Nephritis, however, often occurs acutely and is produced by all absorbable irritants.

Rabbits can be conveniently used for the production of experimental nephritis. The presence of albumin, casts, and sugar should be sought for in the urine, and the kidneys should be hardened, stained, and examined histologically.

EXERCISE XX.—(DEMONSTRATION) URANIUM HYDROPS

(REPORTER V, A)

Inject hypodermically into rabbit 5 mg. (1 c.c. of 5 : 1000) of Uranium Nitrate. Repeat daily for three days.

EXERCISE XXI.—(OPTIONAL) OTHER NEPHRITIC POISONS

Arsenic—Mainly Glomerules.—Inject hypodermically 10 mg. per kg. of Potassium Arsenate: the urine becomes albuminous in ten minutes. The glomeruli are dilated, filling Bowman's capsule. The epithelium of the convoluted tubules is affected to a varying degree; the straight tubules are not involved.

Aloin—Mainly Epithelium of Convoluted Tubules.—Inject hypodermically 2 c.c. per kg. of a 5 per cent. solution; repeat for two or three days. The action is practically limited to the convoluted tubules.

Chromates—As Aloin.—Inject hypodermically 30 mg. per kg. of Potassium Bichromate: nephritis is plain in twenty-four hours.

Cantharidin—All Renal Elements.—Inject hypodermically 5 mg. per kg. (dissolved in acetic ether): albuminuria in ten minutes.

Mercuric Chlorid—Mainly Interstitial.—Inject hypodermically 10 c.c. of 1 : 1000 solution daily: albuminuria in two to three days.

Oxalates—Occlusion of Tubules by Crystals of Calcium Oxalate.—Inject hypodermically 0.250 gm. of Ammonium Oxalate into a rabbit.

Chloroform, Phosphorus, Hydrazin.—Fiske and Karsner, 1914, *Jour. Biol. Chem.*, 18, 381.

TECHNICAL REFERENCES

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- Renal Circulation in Nephritis.**—Schlayer and Hedinger, 1907, Deut. Arch. klin. Med., 90, 1.
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- Plasma Proteins.**—Quantitative Estimation, Cullen and Van Slyke, 1916, Proc. Soc. Exp. Biol. Med., 13, 197.
- Elastometer for Measuring Edemas.**—Schade, 1912, Zs. Exp. Path. Ther., 11, 369; A. B. Schwartz, Arch. Int. Med., 17, 396; Maver and Schwartz, *ibid.*, 459.

(G) REFLEX EFFECTS OF IRRITANTS; ARSENIC ON CIRCULATION

Sensory reflexes produce marked changes in the circulation and respiration. The effects differ for each region, but are naturally least marked where sensation is least developed, *i. e.*, in the gastro-intestinal tract.

EXERCISE XXII.—(OPTIONAL) TRIGEMINAL—VAGUS (KRETSCHMER)
REFLEX—CHLOROFORM, AMMONIA

Feel pulse of rabbit. Blow into nostrils the vapor of chloroform; and when the animal has recovered, the vapor of ammonia: marked slowing or temporary arrest of the heart.

EXERCISE XXIII.—(DEMONSTRATION) IRRITANTS ON BLOOD-PRESSURE
AND RESPIRATION

(REPORTER V, A)

Morphinize dog (20 mg. = $\frac{1}{2}$ c.c. of 4 per cent. per kg.). Etherize. Insert tracheal cannula with T piece and connect with tambour for respiratory tracing. Connect carotid artery for blood-pressure tracing; femoral vein for injection. Remove ether. Start slow tracings.

Experiment 1. Tracheal Irritation.—Blow ammonia vapor into trachea: little or no effect.

Experiment 2. Laryngeal Irritation.—Blow ammonia vapor into mouth so as to reach larynx: marked disturbance of respiration and blood-pressure.

Experiment 3. Irritants in Mouth.—With a pipet flood the mouth with 5 per cent. acetic acid: marked disturbance (mainly from larynx).

Experiment 4. Corrosives in Stomach, Intestines, and Peritoneum.—Make small opening into abdomen, expose stomach and intestines, and with a pipet apply concentrated nitric acid successively to the interior of the stomach and intestines, and to the visceral and parietal peritoneum: usually but little effect.

EXERCISE XXIV.—(DEMONSTRATION) ARSENIC ON CIRCULATION

(REPORTER V, A)

Inject intravenously 50 mg. per kg. of Arsenate of Sodium (1 c.c. per kg. of 5 per cent.): the intestines show capillary congestion and become filled with fluid (paralysis of the capillary walls). The blood-pressure falls, but

rises at once if the aorta is temporarily compressed, showing that the cardiac muscle is not injured (except by larger doses). Stimulation of the sciatic or splanchnic nerve also causes a rise. Kill the animal and examine the gastro-intestinal lesions.

QUESTIONS

- (a) Which surfaces give the most, which the least, reflexes with irritants?
- (b) What therapeutic use could be made of these reflexes?
- (c) How can the danger of reflex arrest of the heart by chloroform inhalation be minimized?
- (d) What effects has arsenic on the circulation?
- (e) Is the effect due primarily to depression of the heart? Of the vaso-motor center? What then?
- (f) Describe the autopsy lesions of arsenic.
- (g) Describe the autopsy lesions of nitric acid.

TECHNICAL REFERENCES

Irritant Reflexes.—Heidenhain and Gruetzner, 1877, Arch. ges. Physiol., 16, 55; Sollmann, 1907, Amer. Jour. Physiol., 20, 74.

CHAPTER XL

CONVULSANTS AND TREATMENT OF POISONING

(REPORTER III, D)

(A) CONVULSANTS

Introduction.—The effects of convulsant poisons are very similar in frogs and in mammals. They can be localized by the same methods, but the technic is naturally more difficult in the higher animals. Only the symptoms will be studied in this exercise. The seat of the action is the same as in the frog.

Spinal convulsants produce increased reflex excitability, and then tetanic opisthotonus. Strychnin is the principal example; caffein belongs to the same group.

Medullary convulsants produce clonic spasms with tendency to emprosthotonus. Nicotin and hydrocyanic acid belong to this group. They act by producing asphyxia, which is the direct cause of the convulsions. Veratrin, camphor, picrotoxin, ammonium, and some others act directly on the centers.

Cerebral convulsants act on the motor areas. They produce rhythmic twitchings of muscles (choreiform contractions) or epileptiform spasms. These are sometimes seen in morphin poisoning. They are also produced by absinth.

Psychic excitants produce constant motion, but of a purposive type, plainly due to excitement. The movements may assume various types: there may be simply an increased vivacity, as with atropin; or the animal may become maniacal, as sometimes with cannabis; or it may run constantly in a circle.

The central action may also remain localized in certain *definite centers*. Small doses of caffein, for instance, cause an increase of psychic activity

and tendency to wakefulness. Apomorphin acts mainly on the vomiting center; the antipyretics on the temperature. Drugs may also stimulate the vasomotor, vagus, or respiratory center, etc.

The action of convulsants on mammals is often not sharply localized, but involves different centers in succession, generally from the brain downward. Cocain, phenol, and asphyxia are examples.

It will be noted, in the following experiments, that the stimulation is generally followed by depression.

TECHNICAL REFERENCES

Stimulation of Motor Areas, Stewart, 962; **Tigerstedt**, 3,4, 107; **Operations on Brain**, *ibid.*, 79.

OBSERVATIONS

Observe the respiration, general behavior, reflexes, and the onset and type of the convulsions, and time of death.

EXERCISE I.—(GROUP II, A) STRYCHNIN HYPODERMICALLY

(Spinal convulsions.) Administer hypodermically to cat a fatal dose of strychnin, 0.75 mg. ($\frac{3}{4}$ c.c. of 1 : 1000) per kg.: increased reflexes, increased respiration; convulsions, first on stimulation, soon spontaneously; symmetric, first clonic, then tetanic. Respiration arrested during spasms by fixation of muscles; asphyxial symptoms: dilated pupils, cyanosis. Depression between convulsions. Convulsions start in from fifteen minutes to one and one-half hours. Death occurs in from thirty minutes to three hours. Make a sketch-drawing of the tetanic animal.

EXERCISE II.—(GROUP III, B) STRYCHNIN BY STOMACH

Administer by stomach-tube to cat a fatal dose of Strychnin, 1 mg. (1 c.c. of 1 : 1000) per kg.: effects as in Exercise I, but usually rather slower.

QUESTIONS

- (a) Describe the course of strychnin poisoning.
- (b) Is there as much difference in the toxicity, by stomach and hypodermically, as was observed with rabbits (Chapter XXXVIII)?

EXERCISE III.—(GROUP I, A) CAMPHOR CONVULSIONS (CEREBRAL AND MEDULLARY)

Experiment 1.—Administer by stomach-tube to cat or rabbit Camphor, 2 gm (10 c.c. of 20 per cent. in oil) per kg.: convulsions occur in about half an hour or later. They are violent, but asymmetric and irregular. Try whether they can be controlled by inhalation of chloroform. They usually run a long course.

Questions.—How do camphor convulsions differ from those of strychnin?

Experiment 2. (Optional) Camphor Toxicity Modified by Method of Administration.—In guinea-pigs dry camphor by mouth is fatal with a dose of 0.14 to 0.18 gm. per 100 gm. It is less toxic when dissolved in oil. Hypodermically, an oily solution is also less toxic than a solution in alcohol or water; but the oily solution is more toxic hypodermically than by mouth. Peritoneal injection is more toxic than hypodermic, the oily again being the least effective (Cairis, 1914, *Jour. Pharm. Chem.*, 10, 224).

EXERCISE IV.—(GROUP I, B) TREATMENT OF EPILEPTOID (CAMPHOR) CONVULSIONS BY BROMID

(Adapted from Januschke and Inaba, 1913, Zs. exp. Med., 1, 129.)

On morning of previous day administer to cat or rabbit by stomach-tube Sodium Bromid, 2 gm. per kg. (10 c.c. per kg. of 20 per cent.). Repeat at six-hour intervals, giving the last dose an hour before the laboratory period. Administer Camphor to this bromid-cat, as in Exercise III. Compare the results. Calcium also suppresses the convulsions (Januschke and Hirsch, 1913, Ther. Mon., 27, 777).

QUESTIONS

- (a) Describe the bromid symptoms.
- (b) Record the camphor results.
- (c) How does the bromid suppress the epileptic convulsions?

EXERCISE V.—(OPTIONAL)

Experiment 1. Veratrin (Stimulation of Medulla).—Inject hypodermically into a rabbit 1 mg. per kg. of Veratrin salt (1 c.c. per kg. of 1% per cent.). Repeat in twenty minutes, if necessary: salivation, inco-ordination, irregular convulsions, animal jumps straight up ("bucks"). Paralytic condition. If death should occur, the respiration stops before the heart. (The commercial samples of veratrin vary considerably in their activity, and it may therefore be difficult to hit upon the proper dose which is required to produce the "bucking.")

Experiment 2. Absinthe (Epileptic Cerebral Convulsions).—Inject 0.03 to 0.05 of Absinthe Essence per kg.

(B) TREATMENT OF POISONING

Introduction.—The main features of the treatment of poisoning consists in:

- (1) Chemic precipitation, neutralization, or destruction of the poison.
- (2) Removal of the poison.
- (3) Physiologic antidotes.
- (4) General supporting measures.

All treatment must be as prompt as possible.

(1) *Chemic Antidotes.*—These have been discussed in Chapter XVI, which should be consulted.

(2) *Removal of the Poison.*—This is accomplished by washing, emesis, lavage, catharsis, and diuresis.

(3) *Physiologic Antidotes.*—The effects of depressant drugs are counteracted by stimulants, and vice versa. It must be remembered, however, that the action of stimulants passes readily into depression, which would increase the danger. Antidotes should therefore be given in rather moderate doses. It should also be borne in mind that physiologic antidotes remove only the symptoms, and not the action of the poison. They are therefore useful only when the symptoms are a direct source of danger. In the case of strychnin, for instance, death is due to the direct depressant action of the drug, aided by the exhaustion consequent on the convulsions. Chloral, curare, or artificial respiration, by preventing the convulsions, are able to save an animal from several times the fatal dose, but they are quite ineffective against doses sufficiently large to kill by the direct depressant action of the poison.

(4) *General Supporting Measures.*—The immediate cause of death with most poisons consists in failure of the respiration. This should

be carefully watched and supported by hot coffee. Should this prove insufficient, artificial respiration must be instituted, and this before the natural respiration has ceased. The patient should be kept warm. Pain (from corrosives, etc.) should be controlled by morphin or the local use of cocain.

The use of antidotes is well illustrated by Strychnin, as in the following exercises.

TECHNICAL REFERENCES

Saline Infusion on Excretion of Toxic Substances.—Lenhartz, 1899, Deut. Arch. Klin. Med., 64, 189.

Vivification.—Abel, Rowntree, and Turner, 1914, Jour. Pharmacol., 5, 275; MacCallum and Lambert, 1914, Soc. Exp. Biol. Med., 11, 78.

EXERCISE VI.—CHEMIC ANTIDOTES

Experiment 1. (Group IV, B) Strychnin and Permanganate.—Administer Strychnin by stomach-tube as in Exercise II. Follow within five minutes by Potassium Permanganate, 15 c.c. of 1 per cent. per kg. Compare the results.

Experiment 2. (Group V, B) Hydrocyanic Acid and Permanganate.—Administer to cat, by stomach-tube, Hydrocyanic Acid, 2 mg. (2 c.c. of 1 : 1000) per kg. (twice fatal dose). Follow this *at once* with Potassium Permanganate, 15 c.c. of 1 per cent. per kg. The animal usually shows severe symptoms, but survives.

QUESTIONS

- (a) Report the results.
- (b) What is the mechanism of the action of permanganate?
- (c) What would interfere with its usefulness?

EXERCISE VII.—(GROUP V, A) ADSORBENT ANTIDOTES (STRYCHNIN AND CHARCOAL OR CARAMEL)

Administer Strychnin by stomach-tube as in Exercise II. Follow at once with a suspension of 25 gm. of Charcoal or of 25 gm. of Caramel. Compare the results with Exercise II.

QUESTIONS

- (a) Report the results.
- (b) How do the charcoal and caramel act?
- (c) How could their efficiency be increased?

TECHNICAL REFERENCE

Charcoal as Antidote.—O. Adler, 1912, Wien. Klin. Woch., 25, No. 21.

EXERCISE VIII.—(GROUP IV, A) EVACUATION (STRYCHNIN AND LAVAGE)

Administer Strychnin by stomach-tube as in Exercise II. Five or ten minutes later wash the stomach. Compare the results.

QUESTIONS

- (a) Report the results.
- (b) Would lavage be of much use after convulsions have set in?

EXERCISE IX.—(GROUP II, B) ARTIFICIAL RESPIRATION AND STRYCHNIN

Administer Strychnin hypodermically as in Exercise I. When the animal becomes convulsive, start artificial respiration. The convulsions are suppressed. Note that they return if the respiration is intermitted. Continue the respiration until the animal is out of danger. Compare the results with Exercise I.

QUESTIONS

- (a) Describe the effect of artificial respiration on strychnin poisoning.
- (b) Explain the effect.
- (c) Should the artificial respiration be applied only during the convulsions, or how?

EXERCISE X.—(GROUP III, A) PHYSIOLOGIC ANTIDOTE (STRYCHNIN AND CHLORAL)

Administer Strychnin hypodermically as in Exercise I. Follow this at once by Chloral, 0.25 gm. (10 c.c. of 2.5 per cent.) per kg., by stomach-tube. (This dose produces light coma in normal animals.) Compare the results with Exercise I.

QUESTIONS

- (a) Describe the results.
- (b) How does the chloral act as antidote?
- (c) Would it be useful in other convulsions?

CHAPTER XLI**RESPIRATION (AND BLOOD-PRESSURE)**

Introduction.—The respiratory center may be stimulated or depressed by the direct or reflex action of drugs; or indirectly, for instance, by changes in the circulation, by acidosis, etc. The respiratory movements may also be altered by local changes in the lungs, air tubes, pleura, respiratory muscles and nerves, etc.

TECHNICAL NOTES ON METHODS OF STUDYING THE RESPIRATORY MOVEMENTS

The present chapter will deal mainly with modifications in the respiratory movements, their rate and amplitude, etc. These may be observed and counted directly, or they may be recorded by registering the excursion of the chest walls or diaphragm or the passage of air from the lungs or pleura.

Respiratory Tracings.—These may be taken on a separate drum, moving at the same speed as that used for recording the blood-pressure. The levers, etc., are adjusted so that the excursion of the normal respiration has a height of $\frac{1}{4}$ to 1 inch on the drum. The tracing should be marked to show whether inspiration corresponds to the upstroke or downstroke. Several methods will be described; none is universally satisfactory.

1. Trachea-tambour Method.—This is the simplest method, commonly used in anesthetized animals. The tracheal cannula is connected by wide tubing with a large T piece. The second limb of the T bears a short piece of tubing which can be narrowed by a screw-clamp. The third limb

is connected with the recording tambour. The screw-clamp is adjusted so that the lever-point makes the desired excursion. In place of the screw-clamp a hole may be cut in the tubing, which can be partly occluded by a piece of glass-rod inserted through the free end (Fig. 46). If the anesthetic



Fig. 46.—Trachea-tambour method.

is to be given, the open end of the T tube is inserted into the mouth of the anesthetic bottle (not immersed).

This method has the advantage of simplicity and is not disturbed by movements of the animal. It suffices to register the rate and changes in

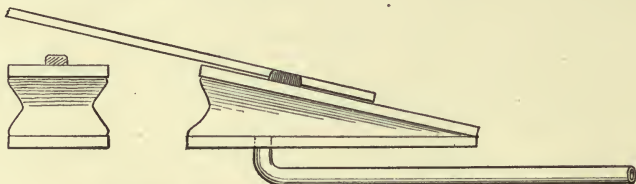


Fig. 47.—Organ-key bellows recorder. Actual size.

the depth of the respiration; but, because of the escape of air, it becomes inaccurate if the respirations are slow or prolonged.

Recording Tambours.—The cheapest form consists of a home-made organ-key bellows (Fig. 47), the sides of very thin leather or gold-beater's skin. A 3- or 4-cm. Marey's tambour answers well. The 3-cm. Brodie bellows (made by C. F. Palmer, 6 Upper Tulso Hill, London, N. W.) is the most delicate. All bear a straw and writing point about 6 inches long.

2. Trachea-bottic-tambour Method.—This avoids the inaccuracy of the preceding method by interposing a large closed bottle into which the animal breathes while the record is taken, but it introduces the complication of more or less asphyxia.

The arrangement is explained by Fig. 48. The bottle should be as large as possible (a 5-gallon glycerin can or large jug answers). The connection between the trachea and bottle should be as short and wide as possible. The vent is closed whenever tracings are taken, and opened between the tracings. The greatest care must be used to avoid asphyxia. It is advisable to disconnect the bottle occasionally and blow air through it with bellows.

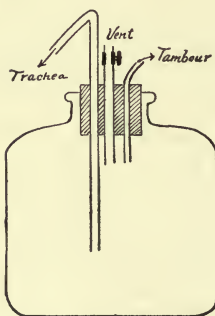


Fig. 48.—Respiration bottle.

3. Mask-tambour Method.—The air may also be taken from a mask fitting air-tight over the mouth and nose and provided with a T piece, as in the first method. A Henderson "tennis-ball cardiometer" is very satisfactory.

4. Nasal-tambour Method (Unanesthetized Rabbit).—This corresponds to the first method, except that a cannula, expanded into an olive-shaped bulb at the tip, is inserted into a nostril of the animal, and fixed with adhesive plaster, if necessary, and connected with the tambour (Wolff, 1913, Arch. exp. Path., 74, 299).

5. Double Tambour Method (Stethograph).—This can also be used for non-anesthetized animals. A large tambour or other elastic reservoir is tied firmly to the chest or abdomen. Its interior is connected to a recording tambour, with the interposition of a T piece, by means of which the tambours can be moderately distended.

The receiving tambour may be given various forms. An efficient instrument may be made by cutting off the top of a pound ether tin a centimeter below the rim, tying a rubber membrane over this, and closing the stopper opening with a perforated cork, bearing a glass tube.

The sleeve of the sphygmomanometer can be wound about the chest and connected with the recording tambour; or a piece of bicycle tire will answer the purpose.

6. Lever Methods.—In these the motion is transmitted to an ordinary muscle-lever. This may be done (1) by taking a stitch through the skin and tying the string to the lever. (2) A small incision may be made through the skin and muscle, on the right side, about the lower edge of the diaphragm; the end of a glass rod or the bowl of a teaspoon is inserted between the liver and diaphragm and the handle connected with the lever. (3) A knitting needle may be thrust directly into the liver through the skin (danger of hemorrhage!). (4) A special lever may be used, bearing a rod which rests on the chest and abdomen. This does not require anesthesia. It is well adapted to obtaining tracings of the Cheyne-Stokes respiration in deep anesthesia. The animal must be immobilized in all the lever methods.

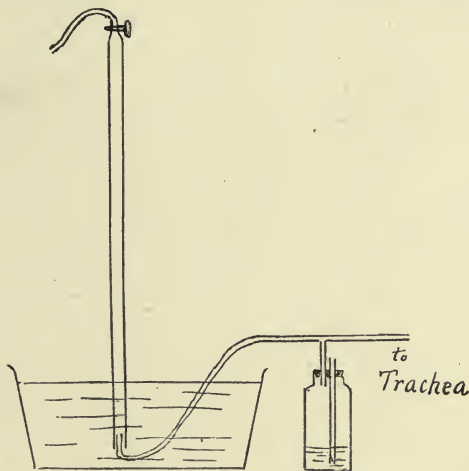


Fig. 49.—Diagram of Dreser spirometer.

7. Respiratory Plethysmograph for Entire Rabbit.—This is described by Cushny, 1913, *Jour. Pharmacol.*, 4, 363; and a simpler form by Cushny and Lieb, 1915, *ibid.*, 6, 451.

8. Pleural Cannula Method.—In this the air is obtained from a flanged cannula in the thoracic wall.

9. Spirometer Methods.—These measure the total volume of air breathed (Fig. 49). The expired and inspired air are separated by the valve. *Gas-meters* may be used instead of the spirometer.

TECHNICAL REFERENCES ON RESPIRATION

- General.**—Kobert, *Intox.*, 1, 202, 243; Stewart, 293.
Observation and Recording.—Tigerstedt, 2.2, 3; Heinz, 2, 427, 433.
Bellows Recorder.—Locke, 1908, *Quart. Jour. Exp. Physiol.*, 1, 367.
Respiration Valves.—Guthrie, 1911, *Amer. Jour. Med. Assoc.*, 57, 887.
Spirometer.—Dreser, 1889, *Arch. Exp. Path.*, 26, 253; *Arch. ges. Physiol.*, 1898, 72, 494; Impens, 1899, *ibid.*, 78, 529.
Gas Meters.—Tigerstedt, 1.3, 144; for small quantities, Y. Henderson, *Amer. Jour. Physiol.*, 25, 385, 1910.

Carbon Dioxid Test for Respiratory Excitability.—A. Loewy, 1890, *Arch. ges. Physiol.*, 47, 601; *in man*, Lindhard, 1911, *Jour. Physiol.*, 42, 337; Y. Henderson (holding of breath as index of acidosis), 1914, *Jour. Amer. Med. Assoc.*, 63, 318.

Respiration Experiments on Man.—Y. Henderson, 1914, *Jour. Amer. Med. Assoc.*, 62, 1133; Higgins and Means, 1915, *Jour. Pharmacol.*, 7, 1.

Respiratory Metabolism.—Tigerstedt, 1, 3, 71; Abderhalden, 3, 1143; *Man*, *ibid.*, 7, 452; 8, 529; *Alveolar Air*, Comparison of methods, Boothby and Peabody, 1914, *Arch. Int. Med.*, 13, 497; *Microspirometer* (small organisms), Thumberg, 1905, *Skand. Arch. Physiol.*, 17, 74.

Alveolar Ventilation and CO₂ Tension.—*Man*, Higgins and Means, 1915, *Jour. Pharmacol.*, 7, 1; *Animals*, Macht, 1915, *ibid.*, 7, 339.

TECHNICAL NOTES ON METHODS OF RECORDING THE ARTERIAL BLOOD-PRESSURE

The usual methods of recording the blood-pressure of anesthetized animals consist in connecting the carotid (sometimes femoral) artery with a manometer which writes on a revolving cylinder (kymograph). The general arrangement is shown in Fig. 50.

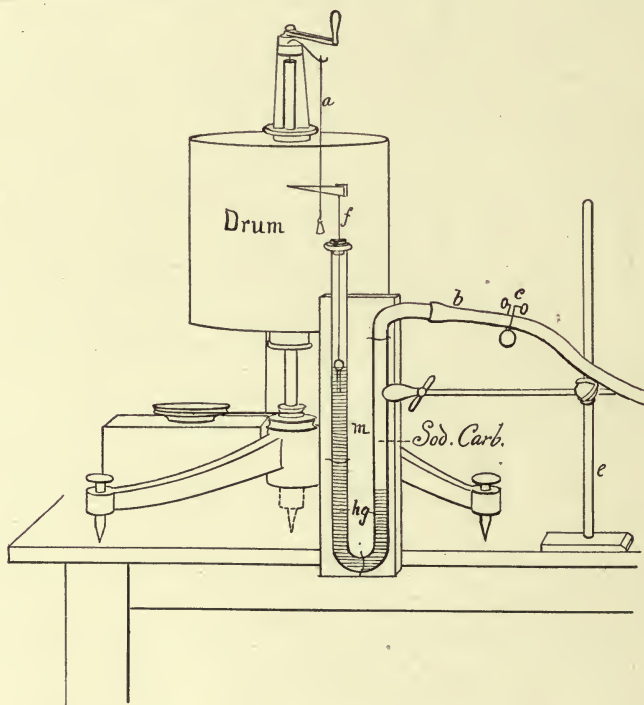


Fig. 50.—Arrangement for taking a blood-pressure tracing (Stewart): *m*, Manometer; *hg*, mercury; *f*, float armed with writing-point; *a*, thread attached to a wire projecting from the drum and supporting a small weight; the thread keeps the writing-point in contact with the smoked paper on the drum; *b* is a strong rubber tube connecting the manometer with the artery; *c*, a pinch-cock on the rubber tube, which is taken off when a tracing is to be obtained.

Taking an Ordinary Blood-pressure Tracing.—The manometer (Fig. 51), containing clean mercury, is clamped to the table. A drop of very thin oil is placed on the float. The arterial limb of the manometer bears a T-tube

(not shown in the figure). The horizontal limb is attached to a rubber tube (which will connect with the artery). A screw-clamp and a strong pinch-cock are placed on this tube. The vertical limb of the **T** is connected with a bulb, placed 4 feet above the table, and filled with half-saturated magnesium sulphate solution. *The stop-cock between the manometer and the magnesium must always be kept closed when the artery is open*, else the solution will reach the heart and speedily kill the animal.

A drum is smoked and adjusted to the manometer with a guide-thread. The opening of the arterial tube is raised (on a tumbler) to the position which it would occupy in the animal. The pinch-cock is removed and the screw-cock is opened. The magnesium cock is now opened, filling the connections air-free. It is then shut off. This gives the zero pressure in the manometer. The drum is adjusted so that the writing-point of the manometer is about an inch from the bottom. A signal magnet is adjusted at the same point. This traces the zero pressure abscissa line.

The artery tube is now clamped, and the magnesium cock opened until the pressure has risen. The magnesium cock is then closed. The drum is adjusted so as to move about 2 cm. per minute, and a minute's revolution is marked off on the abscissa. This serves as a measure of the time for the whole tracing. (This is not necessary if a time signal is used.) If a respiratory or other tracing is also to be taken in the same drum, the writing-point is adjusted on a vertical line from the manometer-point, about $1\frac{1}{2}$ inches from the top of the drum.

The artery cannula may now be filled with magnesium and connected with the artery tube (making sure that the magnesium cock is closed) and the tracing started. The screw-clamp on the artery tube is tightened until the excursions are of moderate degree (3 to 10 mm.). This gives a more accurate record of the mean pressure, and also prevents the excessive flow of magnesium into the artery. Injections, etc., are marked with the signal.

A normal tracing should always be taken before the drug is injected. Tracings should also be taken during the injection and whenever any interesting phenomenon occurs. It may be advisable to stop the drum between these periods, especially if a fast speed is used. This is not often necessary in using the slow gear and the 10×2.2 cm. vane of the Harvard kymograph, the most generally useful for pharmacologic work. Only a single round of tracings should be taken on each paper. (It is sometimes desirable to take both a slow and a fast tracing at the same time, joining two manometers to the same carotid by a **T** piece and using two kymographs; this is especially instructive with digitalis and aconite. The slow tracing is made continuous, while the fast tracing is only taken at intervals.)

If clotting occurs, i. e., if the manometer ceases to pulsate, the artery is clamped, the cannula detached and cleaned with a feather, and the artery tube is flushed with magnesium.

The actual blood-pressure may be read from the tracing, being twice¹ the vertical distance between the tracing and the abscissa.

TECHNICAL REFERENCES ON GENERAL TECHNIC OF BLOOD-PRESSURE EXPERIMENTS

Abderhalden, 5, 125; Heinz, 1, 845; 2, 158; Kobert, Intox., 1, 225.

Comparative Vasomotor Reactions in Different Arteries, Gunning, 1916, Amer. Jour. Physiol., 41, 1.

¹ Since the tracing represents the excursions in but one limb of the manometer, the mercury in the other limb is, of course, changed by the same amount. The pressure corresponds to the difference between the two limbs, i. e., to twice that in one limb.

MANOMETERS: MERCURY MANOMETER

This consists of a glass tube, bent as shown in Fig. 51. No. 9 tubing is used for dogs, No. 7 for rabbits. The straight limb is about 10 inches high. It may be surmounted by a T-tube for connection with the magnesium. The tube is mounted on a small board. A cleat may be screwed to the back of this board, about its middle, projecting an inch on one side. This is clamped to the table. It should be leveled so that the vertical tube is plumb. The board also bears a millimeter scale, with arbitrary zero point. The manometer is filled about one-half with mercury. The bent limb is filled with 25 per cent. magnesium sulphate solution, and connected with a stiff rubber tube long enough to reach to the carotid cannula. This tube is closed with a pinch-cock (or a lead tube and metal stop-cock may be substituted, but with little advantage). The connecting tube is also filled with magnesium solution by means of a long-pointed pipet. The pressure in the manometer is now raised to about the blood-pressure of the animal (say 120 mm.). This may be accomplished simply by forcibly blowing into the rubber tube, clamping near the manometer, and again filling the tube, or the tube may be connected by a T piece with a perfusion bottle filled with a magnesium solution and raised to the desired level.

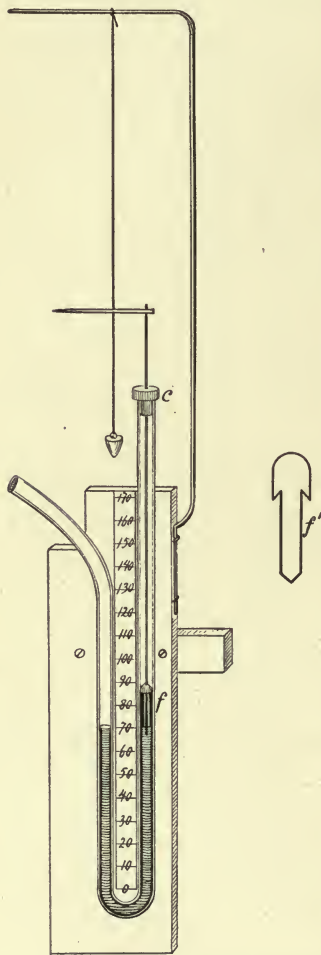


Fig. 51.—Mercury manometer, one-quarter actual size; f' , section of float, actual size (Brown).

mean-pressure. This calculation is scarcely necessary in most cases—a little judgment will enable one to draw the line of mean-pressure approximately without their aid.

The excursions of the manometer with each heart-beat correspond to the pulse-pressure. (The excursions of one limb, as seen on the tracing, must be multiplied by 2.)

The mercury manometer gives only a rough indication of this, the results being vitiated by the inertia of the mercury. It also gives a very imperfect picture of the details of the individual pulse-waves. An elastic manometer (e. g., Huerthle's) is necessary for their

For recording the excursions of the manometer the straight limb bears a float, f . This consists of a little cylinder of hard rubber, of the shape and size shown in the figure. It should fit snugly but rather loosely in the tube. It bears a knitting needle, well centered. This again passes through a hard rubber cap, c . At the upper extremity this needle carries a small flat piece of cork, to which the writing style is attached. This may be of parchment paper, celluloid, a needle, or a quill pen. The writing-point should be bent toward the drum. A few drops of engine oil should be placed in the tube of the manometer. The mercury must not mount above the float. The writing-point is held against the drum by a guide, consisting of a silk thread, suspended from a wire, and loaded with a 10-gram weight.

The mean blood-pressure equals the difference between the readings taken at the highest point reached by the mercury in each limb of the manometer. It may also be obtained from the tracing by doubling the distance between the line of zero pressure and the tracing.

This figure for the mean-pressure is only correct if the excursions are small or if the systolic and diastolic variations are of equal duration. If they are not, the excursions may be reduced by a screw-clamp on the rubber tube; or the mean-pressure can be calculated from the tracing. A series of vertical lines are drawn from the abscissa to the tracing, at equal intervals.

The mean length of these equals one-half the mean-pressure.

accurate study. The mercury manometer is especially useful on account of its simplicity and for obtaining the mean pressure. Very good results are obtained by taking simultaneous tracings with both manometers, connecting the mercury with the carotid, and Huerthle's with the femoral.

Technical References on Manometers.—Abderhalden, 5, 130; Tigerstedt, 2, 4, 1.

Mercury, Guthrie, Jour. Amer. Med. Assoc., Nov., 14, 1903.

Optical, Wiggers, 1914, Amer. Jour. Physiol., 33, 384; 1915, Jour. Amer. Med. Assoc., 64, 1305.

Principles of Registration.—Tigerstedt, 1, 4, 51.

Purification of Mercury.—Abderhalden, 3, 560, 563.

Signal Magnet.—This is useful for marking the time of injections, stimulations, etc. The Harvard instrument is efficient. The electromagnet is connected with a battery (which may be placed under the table), with the interposition of a key, which is closed whenever a mark is to be made on the drum. It may be kept closed during the duration of the injection. The writing-point of the signal must be exactly on a vertical line with the writing-point of the manometer.

Injection Signal.—A simple device for recording automatically the beginning and duration of injections is described by Chase and Schlomovitz, 1915, Jour. Pharmacol., 6, 561.

Interpretation of Membrane Manometer Curves.—Pilcher, 1915, Amer. Jour. Physiol., 38, 209.

ANTICOAGULANT SOLUTIONS

Magnesium Sulphate, half-saturated (25 per cent. of crystals) is the most satisfactory solution for dogs and rabbits. It does not answer quite as well for cats, or where large pressure changes are anticipated. Care must be taken, however, that it does not enter the heart, for it causes prompt paralysis of this organ. The danger of this accident is not great unless too high a preliminary pressure has been produced in the manometer. The effects pass off very quickly unless the heart is stopped completely. Should this occur, it is often possible to resuscitate the animal by artificial respiration, injection of normal salt solution, and cardiac massage. (Magnesium sulphate must never be used to fill the connection with the injection buret).

Devices for Lessening the Entrance of the Anticoagulant Solution into the Circulation.—A bulb of about 15-c.c. capacity, shaped as in Fig. 52, may be inserted horizontally, next to the arterial cannula.



Fig. 52.—Magnesium bulb.

Other devices are described by Brooks and Luckhardt, 1915, Amer. Jour. Physiol., 36, 104.

Other Anticoagulant Solutions.—*Carbonate-bicarbonate Solution.*—Sodium bicarbonate, 46 gm.; Sod. carbonate, 71 gm.; water, q. s., 1 liter.

Carbonate Solution.—Half-saturated; quite toxic.

Sodium Citrate.—One per cent.

Sodium Sulphate.—Half-saturated. The Sodium Citrate and Sodium Sulphate are less dangerous, also less efficient.

Leech Extract may be used in the cannula, as well as in the entire animal.

It is sometimes necessary to render the blood of an animal non-coagulable; for instance, in measuring the outflow from veins, or for practising transfusion.

The best method consists in the intravenous injection of *leech extract*. For each kilo of body weight the heads of three leeches are rubbed with sand and 6 c.c. of 0.9 per cent. salt solution. This causes apparently no change in the circulation.

The preparation of more permanent extracts is described in Abderhalden, 2, 900; Tigerstedt, 2, 4, 325; Abel, Jour. Pharmacol., 5, 270. *Merck's Extr. Sangisuga sic.* comes

in tubes of 0.1 gm., corresponding to three heads, and sufficient for 1 kg. of blood. *Hirudin* requires 1 mg. for 5 c.c. of blood.

The same object may be accomplished by the **Lewaschew-Pick method of defibrination**. About 20 c.c. of blood per kg. of animal are drawn from an artery into a porcelain capsule, defibrinated by beating with a glass rod, strained, warmed, and reinjected into a vein. This is repeated every half-hour until the blood yields no coagulum. Six or seven defibrinations are needed for this end. *Peptone* is less certain and causes a considerable fall of blood-pressure; 0.3 to 0.6 gm. of Witte's peptone per kilo are injected intravenously (as 5 per cent. solution).

TECHNICAL REFERENCES ON BLOOD-PRESSURE IN NON-ANESTHETIZED ANIMALS

Brooks, 1910, Jour. Amer. Med. Assoc., 55, 372; Heart, 2, 5; 1915, Amer. Jour. Physiol., 36, 104; Van Leersum, 1911, Arch. ges. Physiol., 142, 377; Trendelenburg, 1913, Zs. exp. Med., 2, 1; Kobert, Intox., 1, 205.

TECHNICAL NOTES ON ORDINARY OPERATIVE ANESTHESIA

Operations Are to Be Made Only Under Complete Surgical Anesthesia.—

The method of anesthesia depends to some extent on the animal (see also Chapter XLII).

The anesthetic may be administered either by inhalation or by injection. Inhalation anesthesia is best adapted to relatively short operations; injections are preferred when the conditions must be kept constant for some time. The combination of both methods is often advantageous.

ANESTHETICS ADAPTED TO DOGS

Morphin-ether Anesthesia.—10 to 20 mg. of Morphin per kg. (hydrochlorid or sulphate, $\frac{1}{4}$ to $\frac{1}{2}$ c.c. per kg. of 4 per cent. solution) is injected hypodermically (before the laboratory time) and followed in half an hour or an hour by the inhalation of ether.

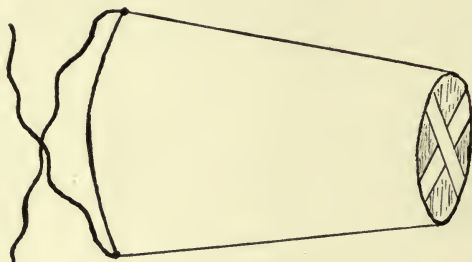


Fig. 53.—Ether cone, about one-half actual size.

If the larger dose of morphin has been used, the ether may usually be withdrawn when the operation is completed, the morphin sufficing to keep the animal narcotized, except when especially painful procedures are employed, when it can be again reinforced with ether. Young dogs should receive relatively less morphin.

Ether Cone for Dogs.—This consists of a conical tin (Fig. 53). The interior of the bottom, which is open except for the cross-pieces, is lined with a small handful of cotton. Two copper wires are fastened behind the ears of the animal to hold the cone in place.

Administration of Ether to Dogs.—The operator kneels over the animal, holding it firmly behind the ears. A tablespoon of ether is poured into the

cone, and this is fastened on the animal and tightened with a towel. More ether is added as needed, and if the animal is not anesthetized in a reasonable time, the holes are occluded with the hand. Complete muscular relaxation is the best sign of adequate anesthesia. The anesthetist must keep his attention constantly on the animal, and regulate the anesthetic and supply air or artificial respiration as needed. During long operations the animal must be kept warm with towels, etc.

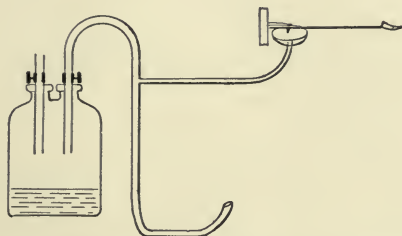


Fig. 54.—Wouff's bottle for giving anesthetic (also arranged for respiratory tracing).

When the trachea has been opened, the tracheal cannula is connected by a tube with a Wouff bottle (250 c.c.) containing cotton moistened with ether. The concentration of the vapor may be varied by the distance of the tube from the ether, or by limiting the intake of air.

Fig. 54 shows this arrangement adapted to respiratory tracings. D. E. Jackson, 1912, Jour. Amer. Med. Assoc., 58, 475, describes a special ether valve.

The ether may also be given by insufflation (Fig. 55; see also Chapter XLII).

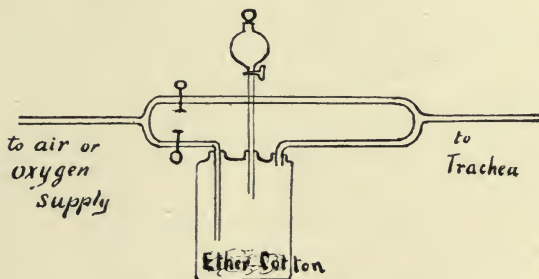


Fig. 55.—Ether by insufflation

Ether may, of course, also be used without morphin, but is much less satisfactory and much more dangerous.

Other inhalation anesthetics may be substituted for the ether, but are less satisfactory for general use.

Chloroform or an *A. C. E. Mixture* (equal parts of Alcohol, Chloroform, and Ether) lower the blood-pressure and are more dangerous.

Ethyl Chlorid is useful for very short operations. It may be given without morphin by spraying it on some cotton placed in the bottom of a tumbler, which is then inverted over the mouth and wrapped with a towel. Better results are secured by giving it in gas form through a special mask. *Nitrous Oxid* is also useful for very short operations.

Grehant Anesthesia.—This is one of the best injection methods, giving a lasting anesthesia. The animal is given a hypodermic injection of 0.01 gm. per kg. of morphin ($\frac{1}{4}$ c.c. per kg. of 4 per cent.), followed in half an hour by 6 to 10 c.c. per kg. (according to age) of the mixture, diluted with water to make a total of 200 c.c., administered by the stomach-tube. The anesthesia is complete in five to fifteen minutes and lasts for eight to fourteen hours. The mixture consists of chloroform, 50 c.c.; alcohol and water, each 500 c.c.

Morphin-chloretone Anesthesia.—This is similar to the Grehant, but cannot be used if the animal is to recover from the operation. The morphin is injected as above. After one to three hours 0.2 gm. per kg. of chloretone, dissolved in a small quantity of alcohol, is injected through a stomach-tube.

Chloral.—This is but little used. The dose is 0.25 to 0.3 gm. per kg. by stomach; 0.1 to 0.15 gm. per kg. by vein.

ANESTHETICS ADAPTED TO CATS

Morphin-atropin-urethane.—This has been the most satisfactory cat anesthetic in this laboratory. The atropin is intended to prevent reflex-vagus stoppage. It cannot be used if the vagus is to be studied. The mixture contains morphin sulphate, 1 gm.; atropin sulphate, 20 mg.; ethyl carbamate, 20 gm.; water, q. s. 100 c.c. Of this solution, 3 c.c. per kg. are administered by stomach or, preferably, by rectum a full half-hour before the operation. The animals appear conscious, but may be tied and operated without resistance or other signs of pain; but it is advisable to give a little ether during the operation.

With a little experience cats can be handled without any danger, but it is safer to wear gloves.

Morphin-chloretone.—Edmunds and Cushing place the animal in a box 35 cm. long, 18 cm. wide, and 18 cm. deep. The box is furnished with a sliding lid. A V-shaped cut is made in the end of the lid and in the corresponding end of the box, so that the animal may be securely clamped in this opening, allowing the head to protrude. The lid is fixed with a nail; 40 to 60 mg. of morphin are injected with a hypodermic syringe into the skin of the neck. This is followed by 0.3 gm. per kg. of chloretone dissolved in alcohol, administered by a stomach-tube.

The chloretone (same dose in oil) may also be injected into the peritoneum.

Ether or Chloroform-ether (Equal Parts).—The animal is placed in a tight box or bell-jar, into which are dropped sponges saturated with the anesthetic until the required degree of anesthesia is procured.

ANESTHETICS ADAPTED TO RABBITS

Morphin-urethane.—Morphin, 5 mg. ($\frac{1}{8}$ c.c. of 4 per cent.) per kg., hypodermically, with ethylcarbamate, 0.75 gm. per kg. by stomach or 0.5 gm. per kg. by rectum.

Or urethane alone, 1 gm. per kg. by stomach or 0.75 by rectum; or *Chloral*, 0.6 gm. per kg. by stomach, 0.3 gm. per kg. per rectum; or *Paraldehyd*, 1 gm. per kg. by stomach; or *Chloretone*, 16 c.c. of saturated watery solution per kg. (often fatal) may be substituted.

The analgesics may be supplemented after fifteen or twenty minutes by light and careful etherization, but this is rarely necessary. Rabbits bear chloroform very badly.

ANESTHETICS ADAPTED TO MONKEYS

Morphin, 30 mg. for small, 60 mg. for large animals, followed by ether.

ANESTHETICS ADAPTED TO SMALL ANIMALS

Mice, guinea-pigs, rats, etc.: Ether.

ANESTHETICS ADAPTED TO FOWL

Paraldehyd, 2 c.c. per kg. by rectum (Edmunds and Roth, 1908); or Atropin, 0.3 mg. hypodermically, followed immediately by ether (Pearl and Surface, 1909, Jour. Amer. Med. Assoc., 52, 382).

DECEREBRATION

This was described in Chapter XXXIV, page 160.

INTRACEREBRAL MAGNESIUM CHLORID

Henderson, Jour. Pharmacol., 1, 199.

SPINAL ANESTHESIA

Tigerstedt, 3.4, 8.

OPERATIVE TECHNIC

Animal Boards.—For convenience in operating the anesthetized animals should be tied to a board. A number of complicated holders are in use, but the one illustrated in Fig. 56 is cheap and answers almost every purpose. It should slope gently toward the feet.

A number of sizes should be on hand for different sized animals (1 by 4 feet for dogs; 8 by 30 inches for rabbits). The cross-piece is made of wire $\frac{3}{16}$ inch in diameter. It is

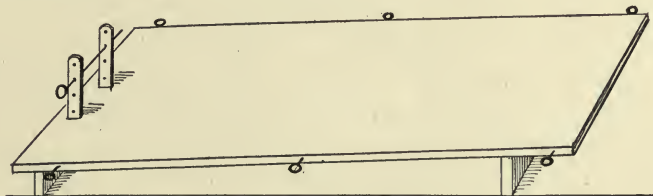


Fig. 56.—Dog board.

pushed back of the canine teeth. A 2-foot piece of stout twine¹ is passed under the neck, behind the ears, the ends are brought forward, wound tightly around the wire, and tied about the mouth. This holds the head very securely. In operating on the neck the front legs should be tied toward the abdomen; in operating on the chest, they are secured toward the head.

In prolonged operations it may be advisable to heat the animal to prevent shock.

Technical References.—*Animal Holders.*—Tigerstedt, 2.4, 11.

Brodie Operating Table.—Pittenger, 116.

Operative Dissections.—The hair of the animal should be well clipped over the field of operation. Scissors (6-inch), curved on the flat, are efficient. The smaller cut hairs are removed by a wet sponge. The wound should be kept as free from blood as possible. This frequently determines the success or failure of a delicate operation. Incisions should be made, if possible, in the median line; the muscles and fasciæ should be separated by blunt dissection. Bleeding vessels are secured by hemostats and tied. Blood is removed by sponging with small pieces of absorbent cotton. Practically no blood should be lost in operating on the neck, groin, or abdomen. The wound may be spread by tenaculi, etc., or by weights attached with a cord to hooks.

¹ India hemp No. 3 for dogs; dauntless flax No. 24 for rabbits.

Operations on the Neck.—The forelegs are tied toward the tail. The structures are most conveniently reached by a median incision, from the lower end of the larynx to near the sternum. The tissues should be divided by layers, keeping to the median line, until the **trachea** is reached. This may be lifted with the fingers and cleaned with the forceps. Tracheotomy is the first step in pharmacologic experiments, as it facilitates anesthesia and artificial respiration. By feeling outward from the trachea, at the bottom of the wound, the **carotid artery** may be felt pulsating. It is lifted to the surface with the fingers, or by turning the edge of the wound outward. The **vagus nerve** in the dog lies in the same sheath as the artery, and must be carefully and gently separated from it. It should never be included in the arterial ligature. In the *dog* the vagus trunk includes the **sympathetic** and **depressor fibers**. These run separately in the *rabbit*, but all in the immediate neighborhood of the artery; they may be recognized by their size, the vagus being the largest, the depressor the smallest. (Illustration in Heinz, I, p. 730.)

Stimulation and Division of Nerves.—Nerves must always be manipulated gently. If it is desired to stimulate or divide the vagus or any other

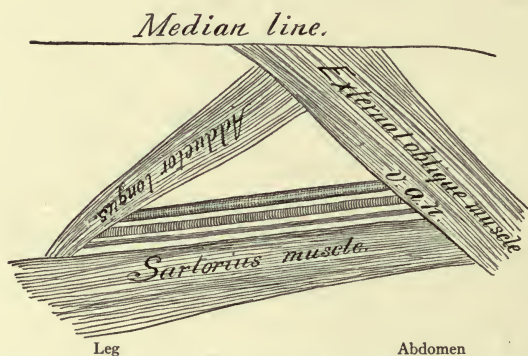


Fig. 57.—Diagram of dissection of femoral vessels of dog (Brown).

nerve later in the experiment, a ligature may be passed under it and the ends knotted. The nerve can thus be easily found and lifted from the wound. In other cases it may be desirable to divide the nerve, securing each end with a ligature. Nerves should always be protected from drying, leaving them in the wound, if possible. In electric stimulation, good contact of the electrodes should be secured. Stimulation of adjacent structures may be prevented by slipping a strip of rubber-dam under the electrodes, or by the use of "shielded" electrodes.

For the dissection of the *Accelerator Nerves*, see Practical Physiology, Beddard, etc.; or Heinz, I, p. 726. A preliminary dissection is indispensable.

The **external jugular vein** is exposed by blunt dissection between the skin and muscle. It offers no difficulty. It may also be reached directly by a skin incision made about the middle of the neck, in a line drawn from the angle of the jaw to the manubrium.

The **thoracic duct** may also be isolated in the base of the neck. It terminates in the left subclavian vein. A practice dissection is necessary.

Exposing the Femoral Vessels.—These may be felt pulsating just below Poupart's ligament on the outer edge of the stiff adductor longus muscle. The artery lies partly behind and external to the vein (Fig. 57). The cannulæ should be introduced as high as possible. As the vessels give off branches in this region, the dissection must be made carefully.

The Sciatic Nerve.—To expose this the hind leg is held up, and an incision is made through the skin in the median ridge of the posterior surface of the thigh. The muscles are separated with the fingers, keeping a little outward from the middle line. The nerve is felt at the bottom of the wound as a hard cord. The animal should be in deep anesthesia when the nerve is handled.

Control of Hemorrhage.—Visible blood-vessels are clamped or tied; capillary hemorrhage is arrested by packing with cotton or Pangawahr Djambi; by pressure with the cut surface of a piece of muscle (V. Horsley, Brit. Med. Jour., July 4, 1914); or by actual cautery.

References.—Tigerstedt, 1,1, 40. The preparation of *hemostatic tissue extract* is described by Hess, 1915, Soc. Exp. Biol. Med., 12, 117; Hirschfelder, 1915, Berl. Klin. Woch., 976.

Technical References on Operative Technic.—Tigerstedt, 1,1, 1; 2,4, 322.

EXERCISE I.—(DEMONSTRATION) MORPHIN, ETC., ON VOLUME OF EXPIRED AIR

(REPORTER I, D)

Arrange a Dreser spirometer (see Fig. 49). If anesthesia is permissible a tracheal cannula should be used. When observations are to be made without anesthesia a mask (cardiometer bulb) is applied. The rabbit is tied on a board or confined snugly in a box, with only the head protruding.

Experiment 1. Effect of Morphin on Normal Rabbit.—Connect apparatus with nostrils. When animal has become accustomed to the apparatus, close the side tube, starting the collection of the expired air and the tracing. Collect the air for one minute; then disconnect from spirometer, but continue respiratory tracing.

Inject hypodermically a therapeutic dose of morphin, 0.5 mg. ($\frac{1}{2}$ c.c. of 1 : 1000) per kg. Repeat observations at intervals.

Experiment 2. Morphin in Hyperpneic Rabbit.—Place a normal rabbit in a box so that it can be heated by hot-water bottles.

Take normal observations. Heat the box until hyperpnea becomes pronounced. Take observations. Inject morphin as in Experiment 1. Take observations.

Remove heat, and when temperature has become normal take observations.

Experiment 3. Toxic Dose of Morphin.—Inject hypodermically into the rabbit of Experiment 1 a toxic dose of morphin, 40 mg. (1 c.c. of 4 per cent.) per kg., and observe results.

Experiment 4. Camphor After Morphin.—Use morphinized rabbit of Experiment 3. After taking normal observation inject into peritoneum camphor 0.1 gm. (0.5 c.c. of 20 per cent. in oil) per kg.

Experiment 5. Caffein After Morphin.—Use morphinized rabbit of Experiment 2. After taking normal observation inject hypodermically caffein, 10 mg. (1 c.c. of 1 per cent.) per kg.

QUESTIONS

- (a) What are the effects of morphin on normal respiration—rate; depth; minute volume; single volume?
- (b) How are these effects modified in dyspnea?
- (c) Under what conditions would morphin be most efficient therapeutically?
- (d) Describe the effects of toxic doses of morphin and state how they differ from therapeutic doses.
- (e) Describe the effects of camphor.
- (f) Describe the effects of caffeine.
- (g) Would these be suitable antidotes for morphin?
- (h) In what pathologic states would they be useful?

EXERCISE II.—(OPTIONAL) BRONCHIAL CHANGES

Dreser's method may be used to study the effect of bronchioconstrictors (pituitary) and bronchodilators (epinephrin, lobelin, etc.) on the respiratory volume. (See Chapter XXXVII.)

EXERCISE III.—(OPTIONAL) RESPONSE OF RESPIRATORY CENTER TO CO_2

(See Loewy, 1890, Arch. ges. Physiol., 47, 601.)

EXERCISE IV.—(GROUP I¹) RESPIRATION OF NORMAL RABBIT

(REPORTER II, D)

Use rabbit with mask-tambour-T-piece method (Tech. Note.), tracing on drum. Observe the effects of the following drugs:

Experiment 1. Auditory Reflex.—Ring bell near rabbit.

Experiment 2. Counter-irritation.—Rub some Capsicum-petrolatum on skin.

Experiment 3. Chloral.—0.5 gm. (20 c.c. of 2.5 per cent.) per kg., by stomach-tube, when depression is pronounced.

Experiment 4. Hypodermic Irritation.—Inject water, 1 c.c. per kg., hypodermically.

Experiment 5. Caffein.—Hypodermically, 10 mg. (1 c.c. of 1 per cent.) per kg.

The respiration increases and the animal may come partly out of the anesthetic (stimulation of the respiratory and other centers).

QUESTIONS

- (a) How does the respiration respond to reflex stimuli?
- (b) Describe the respiratory effects of chloral.
- (c) Describe the respiratory effects of caffeine.
- (d) What measure could be used against respiratory depression?
- (e) Which of these would act most promptly?

¹ Distribution of Work for Exercises IV to VI (Groups I to III):

Student A—Director.

Student B—Weigh animal, give injections.

Student C—Cleaning.

Student D—Reporter; calculate doses.

Student E—General assistant.

Student F—Respiratory tracing.

EXERCISE V.—(GROUP II¹) RESPIRATION OF NORMAL RABBIT

(REPORTER II, D)

Arrange the experiment as in Exercise IV.

Experiment 1. Hypodermic Irritation.—Inject water, 1 c.c. per kg., hypodermically.

Experiment 2. Hypodermic Alcohol.—Inject 50 per cent. Alcohol, 1 c.c. per kg., hypodermically.

Experiment 3. Strychnin, Therapeutic Dose.—Inject hypodermically a therapeutic dose of Strychnin, 0.2 mg. (0.2 c.c. of 1 : 1000) per kg.: increased respiration (stimulation of respiratory center). Reflexes increased (increased excitability of spinal cord).

Experiment 4. Atropin, Therapeutic Dose.—Inject hypodermically Atropin, 1 mg. (1 c.c. of 1 : 1000) per kg.

QUESTIONS

- (a) How does the hypodermic injection of whisky act on respiration?
- (b) Describe the respiratory effect of strychnin.
- (c) Describe the respiratory effect of atropin.
- (d) In what conditions would these be therapeutically useful?

EXERCISE VI.—(GROUP III²) RESPIRATION OF NORMAL RABBIT

(REPORTER II, D)

Arrange the experiment as in Exercise IV.

Experiment 1. Ammonia Reflex.—Blow Ammonia vapor into nostril. Notice respiratory standstill and stoppage of the heart (reflex stimulation of vagus center by irritation of the trigeminal endings). On removing the ammonia the respiration is increased (dyspnea) and the heart resumes.

Experiment 2. Morphin; Therapeutic.—Inject hypodermically 0.5 mg. ($\frac{1}{2}$ c.c. of 1 : 1000) per kg.

Experiment 3. Morphin; Toxic.—Inject hypodermically 20 mg. ($\frac{1}{2}$ c.c. of 4 per cent.) per kg. Respiration becomes slow and shallow (depression of respiratory centers).

Experiment 4. Nicotin.—Inject hypodermically 0.5 mg. ($\frac{1}{2}$ c.c. of 1 : 1000) per kg.

QUESTIONS

- (a) Describe the respiratory effects of irritant vapors.
- (b) Describe the respiratory effects of morphin.
- (c) Describe the respiratory effects of nicotin.
- (d) How could you counteract respiratory depression?

EXERCISE VII.—(GROUP IV³) RESPIRATORY AND BLOOD-PRESSURE TRACINGS

(REPORTER IV, D)

Inject a dog hypodermically with a small dose of Morphin, 10 mg. ($\frac{1}{4}$ c.c. of 1 per cent.) per kg. After half an hour anesthetize with ether.

¹ See foot-note, page 252.

² See foot-note, page 252.

³ Distribution of Work for Exercises VII and VIII (Groups IV and V):

Student D—Director and Reporter; calculates doses; takes notes; prepares report.

Student A—Chief Operator.

Student B—Assistant Operator; weighs animals; gives injections.

Student C—Anesthetist; artificial respiration and resuscitation; cleaning.

Student E—Pulse; blood-pressure tracing.

Student F—Respiratory observation and tracings.

Connect trachea for respiratory tracing (Fig. 54); carotid for blood-pressure, and femoral or vein for injections (Tech. Notes). Determine the effects of the following drugs and procedures:

Experiment 1. Lactic Acid.—Intravenous, 2 c.c. of 0.6 per cent. ($= \frac{N}{15}$) per kg.

Medullary stimulation: increased respiration; slowed heart; moderate rise of blood-pressure.

Experiment 2. Caffein.—Intravenous, 20 mg. (2 c.c. of 1 per cent.) per kg.

Experiment 3. Camphor.—Vein, 0.01 gm. (1 c.c. of 1 per cent. in 40 per cent. alcohol) per kg.

Experiment 4. Reflex Stimulation by Dilation of Anal Sphincter.

Experiment 5. Reflex stimulation by electric stimulation of sciatic nerve with weak, moderate, and strong currents.

Experiment 6. Strychnin, Therapeutic Dose.—Hypodermic, 0.05 mg. (0.05 c.c. of 1 : 1000) per kg.: often no effects. Sometimes slight increase of respiration. Circulation little changed.

Experiment 7. Strychnin, Toxic Dose.—0.25 mg. ($\frac{1}{4}$ c.c. of 1 : 1000) per kg., intravenous; repeated every ten minutes till death. (The dose which is advised is tetanic in normal dogs, but the effect may be diminished by the anesthesia.) Before the onset of the tetanus the respiration is increased, but the circulation is little altered. With the sudden onset of the tetanus the pressure rises abruptly (central vasomotor stimulation), and then falls, with the cessation of the spasm, to a point considerably below normal (central vasomotor depression); the heart is quick during the spasm (inhibition of vagus); slow and strong after (vagus stimulation). The respiration is rapid during the tetanus, depressed in the intervals. Note that the spasms can be brought on by jarring the table or by blowing on the animal. The spasms become successively weaker and the blood-pressure does not rise so high (depression of the convulsive and vasomotor centers). The heart remains rapid (depression of vagus center) but strong. The respiration ceases (paralysis of the center) and the pressure falls. Begin artificial respiration at once: the animal can be kept alive almost indefinitely. The heart and respiration remain fairly good. Note that the pressure varies with the efficiency of the artificial respiration. Let the animal die. Note the early onset of rigor (due to tetanus).

QUESTIONS

Describe the effects of:

- (a) Lactic acid (acidosis).
- (b) Caffein.
- (c) Camphor.
- (d) Dilation of anal sphincter.
- (e) Sciatic stimulation.
- (f) Therapeutic doses of strychnin.
- (g) Toxic doses of strychnin.
- (h) What is the cause of death in strychnin poisoning?
- (i) What are the possible causes of the blood-pressure rise during the strychnin convulsions?
- (j) How could these be distinguished?

EXERCISE VIII.—(GROUP V¹) RESPIRATORY AND BLOOD-PRESSURE TRACINGS

(REPORTER IV, D)

Arrange the experiment as in Exercise VII, but record the respiration by a lever connected with the thorax. The trachea is not opened. Determine the effects of the following drugs and procedures:

Experiment 1. Ammonia Inhalation.—Let the animal breathe Ammonia vapor.

Experiment 2. Ammonium Chlorid.—Vein, 0.15 gm. (15 c.c. of 1 per cent.) per kg.: rate and force of respiration increased, blood-pressure rises; heart variable (stimulation of medullary centers). Respiratory excursions increased (heightened excitability of vagus center). The effects are quite short (rapid elimination) and are not produced by oral administration.

Experiment 3. Mild Asphyxia.—Attach a long tube to the trachea so as to increase the dead space.

Experiment 4. Severe Asphyxia.—Clamp the trachea until the respiration just stops. Revive by artificial respiration. The respiration, especially the inspiratory efforts, will first be increased (dyspnea, stimulation of the respiratory center); then it will be lessened, with rare, gasping, powerful respiratory efforts (depression of center); the blood-pressure rises during the dyspnea (stimulation of vasomotor center), and will then fall; the heart rate is greatly slowed, with typical strong vagus beats (stimulation of vagus center). During the dyspnea the animal makes convulsive movements and the pupils dilate (stimulation of the corresponding centers). The pupils contract again when the paralysis occurs.

Experiment 5. Apnea.—Keep up a brisk artificial respiration for a few minutes. Stop suddenly, and observe that the animal does not breathe for some time, the circulation being good. This *apnea* is due to the fact that there is not enough CO₂ in the blood to stimulate the respiratory center to its rhythmic activity.

Experiments 6 and 7. Strychnin, Therapeutic and Toxic Doses.—See Experiments 6 and 7 of Exercise VII.

QUESTIONS

Describe the effects of:

(a) Ammonia inhalation.

(b) Ammonium injection.

(c) Mild asphyxia.

(d) Severe asphyxia.

(e) Apnea.

(f to k) Strychnin as in Exercise VII.

EXERCISE IX.—(OPTIONAL) PULMONARY EDEMA

Produce pulmonary edema by the methods mentioned below. Observe the blood-pressure, the auscultation changes, and the foaming: Pilocarpin, Muscarin, Methyl Salicylate, intravenously, rabbits (Tyson, Trans. Assoc. Amer. Physic., 21, 175); strong ammonia inhalation. Partial clamping of aorta with overtransfusion of saline. These and other measures are described by Miller and Matthews, Arch. Int. Med., Oct., 1909; R. M. Pearce, *ibid.*, June, 1909; Hallion and Nepper, 1911, Zentr. Bioch. Bioph., 13, 886.

Try the effect of the following **methods of treatment**: Venesection, oxygen insufflation, epinephrin, nitrites, atropin, aspidospermin, strophanthin.

Edema of Perfused Lung.—Modrakowski, 1914, Arch. ges. Physiol., 158, 509, 527.

¹ See foot-note, page 253.

CHAPTER XLII

ADMINISTRATION OF ANESTHETICS ON CIRCULATION AND RESPIRATION

(REPORTERS: C MEMBERS OF EACH GROUP)

Introduction.—Exercises I and II purpose to compare the rapidity and duration of various anesthetics, simple and “combined” with morphin and scopolamin.

Exercises III to VI illustrate the effects of the inhalation anesthetics on respiration and circulation, and the modifications by asphyxia, reflexes, etc., which are liable to arise during anesthesia. The experiments under each exercise are so numerous that it will not always be possible to carry them through; the groups will proceed as far as the condition of the animal and other circumstances permit.

Exercise VII illustrates the treatment of the accidents arising in anesthesia.

TECHNICAL NOTES

Exposure of Kidney and Other Abdominal Organs.—To avoid shock the exposure of the abdominal organs should be limited as much as possible both as to area and time. The organs should be kept warm by packing with cotton, and a can filled with hot water should be kept near the animals. The abdominal incision is made by preference along the linea alba, toward the symphysis pubis. This permits the exposure of *loops of intestine*, of the *spleen*, of the *bladder* and *uterus*, and of the *ureters* where they end in the bladder.

The *ureters* may be seen posteriorly by lifting out the bladder. (Not to be confused with the spermatic cord!)

(In male animals the incision through the skin is carried just to one side of the penis, the superficial veins are ligated and divided, and the dissection is carried along the fascia until the linea alba is reached.)

To **expose the kidneys**, from the median incision, it is necessary to carry this to near the sternum and to make a second, transverse incision along the lower border of the ribs. They may also be reached from the back by an incision about 2 inches from the spine, from the lower border of the rib obliquely downward. If the incision is made to follow the direction of the muscle, there need be very little bleeding. The spleen and intestine may be reached through the same incision.

Oncometry.—See Chapter XXXV.

Artificial Respiration.—This may be maintained in intact animals by alternate rhythmic pressure on the chest and abdomen. Very little force should be used. In operated animals the artificial respiration is maintained through some mechanical apparatus connected with the tracheal cannula.

The simplest device consists in a large *bellows* (15 by 22 inches, exclusive of the handles). This may be arranged for foot power by fastening a spiral upholsterer's “lounge spring No. 2” between the handles. The spout is closed with a cork. An inch hole is bored in the top. This bears a perforated cork, from which a tube leads to the tracheal cannula. A **T** piece is inserted in the course of this tube, the free limb of the **T** being closed when the air is driven into the lungs, and opened when it is expelled. This may be done with the finger, but it is better to employ some automatic device. The **T** piece may be placed directly in the cork of the bellows. The free limb is connected with a rubber tube which is

tied to the handle in such a fashion that it is stepped on and closed when the bellows is compressed (Fig. 58). (The spring may also be placed inside of the bellows.)

R. E. Hall has perfected a simple valve for this purpose (Fig. 59). It consists of a metal **T** piece, with a steel plunger, well fitted and oiled, which is driven up by the bellows and falls back in expiration. The excursions are controlled by short pieces of rubber tubing inserted in the brass.

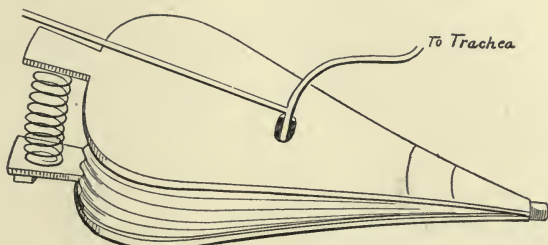


Fig. 58.—Bellows for artificial respiration.

In an emergency the operator can inflate the lungs by blowing into the tracheal tube.

Artificial respiration should be performed at about the rate of the operator's own breathing.

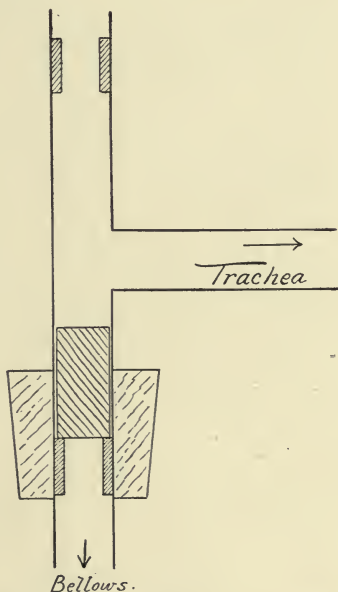


Fig. 59.—Hall's respiration valve. Actual size.

The anesthetic may be continued during the artificial respiration by blowing the air through the Woulff bottle, shown in Fig. 54 (taking care to have the level of the anesthetic so low that it cannot be projected into the tube).

Technical References on Artificial Respiration.—Tigerstedt, 1, 1, 42; Meltzer, 1913, Jour. Amer. Med. Assoc., 60, 1407.

Pumps.—Pittenger, 106; Gates, 1915, Jour. Pharmacol., 6, 611; Hanzlik, 1916, Jour. Lab. Clin. Med.

Interruption of Air-blast.—Gesell and Erlanger, 1914, Amer. Jour. Physiol., 33, Proc. XXXIII.

Pressure Respiration.—Abderhalden, 6, 537.

INSUFFLATION RESPIRATION

A continuous supply of oxygen or compressed air (or glass-blower's bellows) is connected through two T-tubes, provided with stop-cocks so that the air may be passed either through an ether bottle or directly to a catheter with the tips cut off (6 to 7 mm. diameter for dogs; No. 20 French scale for animals of 9 kg.; Nos. 17 to 18 for 7 to 8 kg.).

A dog is anesthetized with morphin and ether. The trachea is opened and the catheter pushed down until it meets a resistance (*i. e.*, when it has entered a bronchus). It is then retracted about 4 cm., which brings it just above the bifurcation of the trachea; the air current is started. (The catheter may also be introduced through the larynx; see Meltzer, 1912, Zentr. Physiol., 26, 204).

The current should be strong enough to have a pressure of 40 to 60 mm. and to distend the lungs moderately. The opening in the trachea should be large enough, and the catheter small enough, so that the air may escape freely. If the stop-cock to the ether is opened and that to the air closed, the animal receives "full ether"; if both cocks are left open, it receives "half ether"; if the ether cock is closed and the air-cock opened, it receives pure air (see Fig. 55, page 247).

References to papers of Meltzer and Auer, Jour. Exp. Med., 1909, 11, 622; Jour. Amer. Med. Assoc., 1911, 57, 521; Zbl. Physiol., 23, 443; *ibid.*, 1912, 26, 204; Jour. Amer. Med. Assoc., 1913, 60, 1407; *ibid.*, 1914, 62, 1547.

CARDIAC TRACINGS

Simple but rather imperfect tracings are obtained with the intact chest by acupuncture; or with opened thorax by simple levers; but specially constructed cardiographs or plethysmographs are needed for reliable tracings.

Acupuncture.—The most convenient method consists in thrusting a knitting needle through the left thorax, a little above the apex-beat, directly into the heart. This causes practically no disturbance in the circulation. Another knitting needle is tied securely to the long limb of a muscle-lever. The two needles are connected by a string. Raising the string on the heart needle or lowering it on the lever needle will increase the excursions. The best results are obtained by adjusting the string in the direction of the movement of the heart needle.

Operation of Exposing the Heart for Cardiographic Tracings.—Dogs serve the purposes of the experiment much better than cats. They should be deeply anesthetized (morphin 20 mg. per kg. followed by ether); a tracheal cannula inserted and the artificial respiration apparatus at hand; a motor-driven bellows with rapid, small excursions gives excellent results; oxygen insufflation may be employed.

The sternum is laid bare by a median incision extending from about the second rib to the ensiform cartilage. Hemorrhage is best controlled

by the cautery, but hemostats may be needed. Artificial respiration is then started. The pericardium is exposed by sawing through the sternum, care being taken to follow the median line. The saw causes much less hemorrhage than the knife. Hemorrhage is again best controlled by the cautery. The sternal edges are separated by hooks attached to the operating board.

After carefully checking all hemorrhage the pericardium is opened and the cardiometer applied.

If the animal is restive, so that the position of the cardiometer is disturbed, curare should be used. A towel or large sponge soaked in warm water should be kept over the thoracic opening between observations.

Technical References.—Tigerstedt, 2, 4, 327.

Tracings From the Exposed Heart.—The thorax is opened under artificial respiration, as described. The heart is exposed, the pericardium is divided, and tracings taken. The opened pericardium may be stitched to the sides of the chest, forming a little hammock for the heart. It is generally advisable to curarize the animal.

Cardiomyographs.—A hook may be inserted in the apex of the heart and connected with an elbow lever. However, this is so easily disturbed by the respiratory and other movements that it is generally unsatisfactory. The difficulty is largely overcome by the Cushny or Guthrie myograph.

Cardioplethysmographs.—These are conveniently constructed from soft-rubber balls, as suggested by Y. Henderson (Amer. Jour. Physiol., 1906, 16, 335): About a third of the ball is cut away and a septum of rubber-dam cemented over the opening. An aperture is burned through this to fit snugly to the auriculoventricular groove (several sizes will be needed for different animals). The opposite pole of the ball is pierced by a glass tube, connected with a large tambour, tracing on a drum. Ordinary tambours may be used for short tracings, but if the heart volume is liable to undergo material changes the small amount of air may lead to serious pressure on the heart. It is therefore better, especially if the total volume changes are to be observed, to employ larger tambours of 10 to 12 cm. diameter. (These can be constructed from the tops of ether cans.)

In applying the apparatus to the heart the pericardium is cut open and the ventricle is slipped into the ball, so that the edges of the rubber-dam fit about the auriculoventricular groove, excluding the auricles. The cardiometer is then connected to the recording tambour. The blood-pressure is a good index of the "fit" of the cardiometer; when the rubber-dam fits too tightly the blood-pressure falls to a low level, while the auricles are distended.

TECHNICAL REFERENCES

Acupuncture.—Tigerstedt, 2, 4, 172.

Cardiographs.—Tigerstedt, 2, 4, 175; Heinz, 1, 846; Stewart, 199; Cushny, 1910, "Heart," 2, 1.

Cardioplethysmographs.—Tigerstedt, 2, 4, 247; Henderson, 1906, Amer. Jour. Physiol., 16, 325; Henderson and Barringer, 1913, *ibid.*, 31, 292; Lehnendorff, 1909 (separate auricles and ventricles), Arch. Exp. Path., 61, 418; Johannson and Tigerstedt, Skand. Arch. Physiol., 1 and 2; Santesson, 1902, *ibid.*, 12.

TECHNICAL REFERENCES ON ANESTHETIC APPARATUS

Hewitt, *Anesthetics*; Kochmann, 1913, Arch. Internat. Pharmacol., 22, 487; Boothby and Sandiford, 1914 (calibration of Waller's gas-balance and Connell's anesthesiometer), Jour. Pharmacol., 5, 369; Jackson, 1915, Jour. Lab. Clin. Med., 1, 1.

EXERCISE I.—(GROUP IV, A¹) ONSET AND DURATION OF ANESTHESIA IN NORMAL RABBIT

Observe the narcosis, respiration, reflexes, and especially the time relations. The animal should be allowed to recover completely between the anesthetics.

Experiment 1. Chloroform Reflex.—Blow chloroform vapor in nostril of rabbit: temporary arrest of heart.

Experiment 2. Cocainization of Nose.—Fill nostril with cotton saturated with 2 per cent. cocaine. From time to time remove the cotton and try reaction to chloroform vapor (or ammonia) until this is abolished.

Experiment 3. Nitrous Oxid Anesthesia.—Let rabbit inhale nitrous oxid through a funnel. Observe effects. Note time of complete anesthesia. Observe color of mucosæ. Are muscles completely relaxed? Remove the gas as soon as anesthesia is complete. Observe symptoms and time of recovery.²

Experiment 4. Chloroform Anesthesia.—Pour about 5 c.c. chloroform on a towel and let rabbit inhale until anesthetized. Observe as in Experiment 3.

Experiment 5. Ether Anesthesia.—Let rabbit inhale about 5 c.c. of ether, and observe as in Experiment 3.

Experiment 6. Morphin.—Inject hypodermically 10 mg. ($\frac{1}{4}$ c.c. of 4 per cent.) per kg. Observe effects during half an hour.

Experiment 7. Ether After Morphin.—Repeat Experiment 5 and compare the results.

QUESTIONS

- (a) What is one of the dangers of early chloroform anesthesia?
- (b) State several means for preventing this.
- (c) Tabulate the relative effects of the various anesthetics as to: Respiration; onset of anesthesia (abolition of pain); persistence of reflexes; muscular relaxation; cyanosis; duration of complete anesthesia after discontinuance of the anesthetic; time to complete recovery.
- (d) Which of the anesthetics would be best adapted for short operations?
- (e) Which would be most dangerous?
- (f) What are the undesirable features of nitrous oxid?
- (g) How do the morphin effects differ from the anesthetics?
- (h) How does morphin modify the course of the anesthesia?
- (i) Would its use be advantageous?

EXERCISE II.—(GROUP IV, B³) ONSET AND DURATION OF ANESTHESIA IN NORMAL RABBIT

Observations as in Exercise I.

Experiment 1. Ethyl Chlorid.—Place some cotton in the bottom of a conical graduate which fits over the face of the rabbit. Pour about 2 c.c. of ethyl chlorid on the cotton and apply to rabbit, wrapping the cone with a towel. Observe effects. Note time of complete anesthesia. Observe color of mucosæ. Are muscles completely relaxed? Remove the cone as soon as anesthesia is complete. Observe symptoms and time of recovery.

¹ Distribution of Work for Exercises I and II, Group IV, A, B:

Students C and F—Director and Reporter; administration.

Students A and D—Reflexes and general symptoms.

Students B and E—Respiration; cleaning.

² Nitrous Oxid as Animal Anesthetic, Dolley, 1914, Jour. Exp. Med., 19, 372.

³ See foot-note No. 1.

Experiment 2. Rectal Ether.—Blow ether vapor into rectum. Observe as in Experiment 1.

Experiment 3. Ether Inhalation.—Administer about 5 c.c. on towel. Observe as in Experiment 1.

Experiment 4. Morphin-scopolamin.—Inject hypodermically, per kg., morphin 10 mg. ($\frac{1}{4}$ c.c. of 4 per cent.) and scopolamin $\frac{2}{3}$ mg. ($\frac{2}{3}$ c.c. of 1 : 1000). Observe effects during half an hour.

Experiment 5. Ether After Morphin-scopolamin.—Repeat Experiment 3, and compare the results.

QUESTIONS

As in Exercise I, questions *c* to *h*, substituting morphin-scopolamin for morphin.

EXERCISE III.—(GROUP I) INHALATION ANESTHESIA TRACINGS

Distribution of Work.—Student C—Director and Reporter; narcosis; observations as below.

Student F—Chief Operator.

Student A—Assistant Operator; weighs animal; calculates doses; gives injections.

Student B—Anesthetist; artificial respiration and resuscitation; cleaning.

Student D—Circulation observations as below.

Student E—Respiration observations as below.

Observations.—Student C—*Narcosis*: Reflexes (corneal); muscular relaxation; pupils; temperature.

Student D—*Circulation*: Pulse, blood-pressure tracing, vascosity (color) of blood. (Set up blood-pressure apparatus, pages 242–246).

Student E—*Respiration*: Count and tracings (set up apparatus, tracheal tambour method, page, 239).

Accidents.—If the animal should stop breathing, resuscitate according to Exercise VII (page 264).

Experiment 1. Induction of Ether Anesthesia.—Observe pulse, etc., respiration, and temperature of normal dog. Pour 15 c.c. of ether into mask and administer by inhalation. Observe behavior of animal and time till complete anesthesia (muscular relaxation).

Operation.—Tie animal to board. Connect carotid for blood-pressure; trachea for respiration; and femoral vein for injection. For respiration, connect T-tube, one limb with tracheal cannula, second limb with anesthetic bottle, third limb with tracing tambour.

Experiment 2. Reflexes Under Light Ether Anesthesia.—Diminish the anesthetic until reflexes are fairly active, but without spontaneous struggling. Aim to maintain this stage. Stretch anal sphincter with artery forceps.

Experiment 3. Insufficient Aëration.—When animal has been brought back to light anesthesia obstruct air passage by partially clamping tracheal tube.

Experiment 4. Change from Light Ether Anesthesia to Chloroform.—Restore the air-way and bring back to light anesthesia. Then change suddenly to chloroform. (In giving chloroform by a mask about 6 to 12 drops are required per minute.)

(Optional) According to Schaefer and Scharlieb, the fall of blood-pressure is practically prevented by adding 10 per cent. of alcohol to chloroform (Hewitt, 471).

Experiment 5. Deep Ether Anesthesia.—Change back to light ether. Concentrate the anesthetic to deep ether anesthesia.

Experiment 6. Reflexes Under Deep Ether Anesthesia.—Maintain a uniform deep anesthesia. Stretch anal sphincter with forceps.

Experiment 7. Insufficient Aëration.—Obstruct air pressure by partially clamping tracheal tube.

Experiment 8. Change from Deep Ether to Chloroform.—Restore air-way. When conditions have reached constant, change suddenly to chloroform.

Experiment 9. Reflexes Under Light Chloroform Anesthesia; and Experiment 10. Insufficient Aëration.—Analogous to Experiments 2 and 3.

Experiment 11. Deep Chloroform Anesthesia; Experiment 12. Reflexes; and Experiment 13. Insufficient Aëration.—Analogous to Experiments 5, 6, and 7.

Experiment 14. Intravenous Ether Anesthesia.—Withdraw chloroform. When reflexes return, inject into vein a saturated solution of ether in N. S., begin with $\frac{1}{4}$ c.c. per kg., and regulate flow so as to maintain an even anesthesia.

(Optional) Chloroform may be also used by vein: 1 c.c. of 0.5 per cent. per kg.; see also Hewitt, 359.

Experiment 15. Chloroform Poisoning.—Stop the ether till reflexes return. Then let animal inhale chloroform till respiration stops.

Experiment 16. Resuscitation.—Follow Exercise VII, page 264.

QUESTIONS

(a) Tabulate the phenomena of light and deep ether and chloroform anesthesia.

(b) What are the chief differences between ether and chloroform?

(c) What is their comparative safety?

(d) How do reflexes (operations, etc.) complicate anesthesia?

(e) How does partial asphyxia complicate anesthesia?

(f) Why is it dangerous to change from ether to chloroform?

(g) Is the change to chloroform safer from light ether anesthesia or from deep ether anesthesia? Why?

(h) What is the comparative safety of intravenous and inhalation ether anesthesia?

(i) What are the phenomena of chloroform poisoning late in anesthesia?

(j) Do the clinical chloroform accidents usually occur in this way?

(k) How may chloroform accidents be treated?

(l) Does the same treatment apply to ether accidents?

EXERCISE IV.—(GROUP II) MORPHIN AND INHALATION ANESTHESIA

This is similar to Exercise III, page 261, except that a morphinized animal is taken, and the kidney volume is also recorded.

Distribution of Work—Observations and Accidents.—As in Exercise III, except that Student F also observes the kidney oncometer.

Experiment 1, a. Morphin.—Observe pulse, respiration, and temperature of normal dog. Inject hypodermically morphin, 10 mg. ($\frac{1}{4}$ c.c. of 4 per cent.) per kg. Repeat observations in half an hour.

Experiment 1, b. Induction of Light Ether Anesthesia.—As in Experiment 1 of Exercise III.

Operations.—As in Exercise III. Also expose kidney and place in oncometer, connected with water-manometer or recording tambour.

Experiments 2 and on.—As in Exercise III.

QUESTIONS

As in Exercise III, page 262.

What differences are introduced by the morphin?

From the comparison of the blood-pressure and oncometer changes deduce whether the circulatory phenomena are vascular or cardiac.

EXERCISE V.—(GROUP V) MORPHIN-SCOPOLAMIN AND INHALATION ANESTHESIA

This is similar to Exercise III, page 261, except that the animal receives morphin and scopolamin, and that the volume of an intestinal loop is recorded.

Distribution of Work—Observations and Accidents.—As in Exercise III, except that Student F also observes the intestinal oncometer and any grossly visible vascular changes in the intestines.

Experiment 1, a. Morphin-scopolamin.—Observe the pulse, respiration, and temperature of normal dog. Inject, hypodermically, morphin 10 mg. ($\frac{1}{4}$ c.c. of 4 per cent.) per kg. and scopolamin $\frac{2}{3}$ mg. ($\frac{2}{3}$ c.c. of 1 : 1000) per kg. Repeat observations in half an hour.

Experiment 1, b. Induction of Light Ether Anesthesia.—As in Experiment 1. of Exercise III.

Operations.—As in Exercise III. Also expose intestines. One loop may be placed in an oncometer.

Experiments 2 and on.—As in Exercise III, page 262.

QUESTIONS

As in Exercise IV (morphin-scopolamin in place of morphin).

EXERCISE VI.—(GROUP III) INSUFFLATION ANESTHESIA AND ANESTHETIC ACCIDENTS

This illustrates the dangers during full anesthesia.

Distribution of Work.—Student C—Director and Reporter.

Student F—Chief Operator.

Student A—Assistant Operator; weighs animal; calculates doses; gives injections.

Student B—Anesthetist; artificial respiration and resuscitation; cleaning.

Student D—Pulse-rate and blood-pressure tracing.

Student E—Cardiograph tracings.

Experiment 1, Morphin.—Observe the pulse and respiration of a normal dog. Inject, hypodermically, morphin 20 mg. ($\frac{1}{2}$ c.c. of 4 per cent.) per kg. Repeat observations in half an hour.

Experiment 2. Induction of Ether Anesthesia.—Administer ether by cone till animal is deeply anesthetized.

Operation.—Tie animal on board. Insert cannulae into carotid, trachea, and femoral vein. Start blood-pressure tracing. Start insufflation (page 258) with half ether. Expose heart and adjust cardioplethysmograph as explained on pages 258, 259. Start tracing.

Experiment 3. Curare.—Inject into vein 3.3 mg. ($\frac{2}{3}$ c.c. of $\frac{1}{2}$ per cent.) per kg.; note blood-pressure changes. If necessary, repeat injection until movements no longer interfere with tracing.

Experiment 4. Excess of Ether.—Change to "full ether." No serious changes (none would occur for several hours).

Experiment 5. Excessive Insufflation Pressure.—Obstruct the outflow from the trachea. Watch the blood-pressure carefully, so that the effect

does not become too severe. Remove obstructions and let conditions return to normal.

Experiment 6. Asphyxia.—Interrupt the air current. Again watch blood-pressure carefully and resume the respiration before it is too late. Let conditions return to normal.

Experiment 7. Chloroform.—Substitute chloroform for ether, with both tubes open ("half chloroform").

Experiment 8. Excess of Chloroform.—Change to "full chloroform." Before conditions become too dangerous return to "half chloroform."

Experiment 9. Asphyxia.—Repeat Experiment 6.

Experiment 10. Myocardial Deficiency.—Inject slowly into vein phenol, 1 per cent., about 5 c.c. (= 50 mg.) per kg. Stop before condition becomes too dangerous.

Experiment 11. Cardiac Dilation from Saline Infusion.—Inject normal saline into vein. Vary speed of injection. Stop when dilation becomes too great.

Experiment 12. Cardiac Failure from Excessive Epinephrin.—Inject slowly into vein a 1 : 10,000 solution of epinephrin until heart stops. If stoppage should not occur, follow with phenol as in Experiment 10.

QUESTIONS

(a) Describe the effects of morphin narcosis, reflexes, pain, pulse, respiration.

(b) Describe the phenomena of the induction of ether anesthesia.

(c) Describe the effects of curare on blood-pressure and heart.

(d) Describe the effects of excess of ether.

(e) Is ether anesthesia by insufflation a safe procedure?

(f) What are the effects and dangers of excessive intratracheal pressure? Explain them.

(g) Describe the effects of obstruction of the air passages under ether and under chloroform.

(h) Describe the phenomena on substituting chloroform for ether.

(i) Describe the effects of excessive chloroform on blood-pressure and heart.

(k) Is the fall of pressure cardiac or vasomotor?

(l) What are the first danger signs?

(m) Why is chloroform insufflation more dangerous than ether?

(n) Describe the effects of phenol on blood-pressure, heart, and motor system.

(o) How does myocardial weakness modify the course of anesthesia?

(p) Describe the effects of saline infusion on the blood-pressure and heart.

(q) Would saline infusion in collapse during anesthesia be beneficial or harmful? Explain.

(r) Describe the effects of epinephrin on blood-pressure and heart.

(s) What is the danger of epinephrin in chloroform collapse?

(t) How would this be guarded against?

EXERCISE VII.—RESUSCITATION

If an animal is killed during anesthesia, proceed *at once* to resuscitation.

Experiment 1. Reflex Stimulation.—Stretch the anal sphincter. If this is not immediately effective, proceed to

Experiment 2. Artificial Respiration.—If this does not succeed promptly, perform

Experiment 3. Cardiac Massage.—*i. e.*, Strong, rapid, *rhythmic compression of the thorax* (rate of at least 80 per minute). This must be done very vigorously. Observe on the tracing that an artificial circulation can be kept up in this manner. If the animal does not revive in two minutes, continue the procedure, but at the same time proceed to

Experiment 4. Intravenous Epinephrin.—1 mg. (1 c.c. of 1 : 1000) washed in with 50 c.c. of N. S. This aids resuscitation by stimulating the heart and blood-vessels. If it does not succeed in two or three minutes, proceed to

Experiment 5. Epinephrin into Carotid.—Connect cardiac end of carotid with pressure bottle containing N.S., placed 3 or 4 feet above the table. With a syringe inject 1 mg. (1 c.c. of 1 : 1000) epinephrin into the connecting rubber tube, while the saline is flowing in. The massage must be continued. The epinephrin, administered in this way, reaches the coronary vessels more directly.¹

QUESTIONS

- (a) Record the success or failure of these different methods of resuscitation.
- (b) Explain their mechanism.

EXERCISE VIII.—(OPTIONAL) MORPHIN-SCOPOLAMIN-ETHER SYNERGISM ON MICE

W. Straub, 1912, *Zs. biol. Technic.*, 2, 277; Fuehner, *Deut. Med. Woch.*, 1913, No. 3.

EXERCISE IX.—(OPTIONAL) RESUSCITATION BY INTRAPERICARDIAL INJECTION

Gunn and Martin, 1915, *Jour. Pharmacol.*, 7, 31.

CHAPTER XLIII

VASOMOTOR DRUGS; TREATMENT OF CIRCULATORY COLLAPSE

(REPORTERS: B MEMBERS OF EACH GROUP)

Introduction (Interpretation of Blood-pressure).—The observation of the blood-pressure is perhaps the most commonly used method for studying changes in the circulation. However, it has certain limitations: the ordinary methods permit only the observation of acute changes. These generally require toxic rather than therapeutic doses. Allowance must then be made for this fact.

In the second place, the changes in blood-pressure give only the sum of the changes produced in the circulation, but do not usually show how and where these effects take place. Changes in blood-pressure may be either cardiac or vascular. The mercury pressure-tracing gives a very imperfect and often erroneous impression of the strength of the heart-beat. It is therefore necessary to distinguish between cardiac and vascular changes by direct experiments. The cardiac effects may be registered with the myo-

¹ Guthrie, 1908, obtained better results by blocking the aorta.

cardiograph. They may also be deduced from the vein pressure, oncometer, or circulation time: these vary generally in the same direction as the arterial pressure if the changes are cardiac; in the opposite direction if they are vascular. However, the conclusions may be deceptive if the drug acts unequally on different vascular areas. Vascular changes may also be distinguished by direct inspection.

Fairly definite conclusions may be drawn from the relation of the systolic and diastolic pressure as recorded by a membrane manometer. The diastolic changes are relatively greater with alterations of the vasomotor tone or heart-rate; whereas the systolic changes are relatively greater with alterations of the cardiac force or blood volume. Therefore, if (A) the diastolic pressure rises relatively more than the systolic, this points to general vasoconstriction or to quickened heart-rate. (B) The diastolic pressure falls more than the systolic: general vasodilation or slower heart-rate. (C) The systolic pressure falls more than the diastolic: cardiac weakening or diminished blood volume (hemorrhage). (D) The systolic pressure rises more than the diastolic: cardiac stimulation or increased blood volume (transfusion). (Pilcher, 1915 Amer. Jour. Physiol., 38, 208.)

If the changes are cardiac it is necessary to distinguish between actions on the cardiac muscle and on the nervous mechanisms, central and peripheral.

Vascular changes may concern the arterial muscle or the vasoconstrictor or vasodilator nervous mechanism. The vasodilator system is only important in a few situations, which are not sufficient to affect the general blood-pressure. It is therefore necessary to consider mainly the vasoconstrictor nerves and the muscle.

Vasoconstriction.—The seat of the stimulation may be:

1. **Central.**—The drug has no effect if it is injected after destruction of the spinal cord. The venous pressure and volume of the leg increases if the drug is injected after section of the sciatic. (Strychnin, caffeine, etc.)

The stimulation may also be *reflex* (counterirritants) or from *convulsions* or *asphyxia*. These must be excluded by curare and artificial respiration.

A direct method for studying the reactions of the vasomotor center is described in Exercise VI.

2. **Peripheral.**—(The drug is effective after destruction of the spinal cord.) The stimulation may be in:

The Ganglia.—The drug does not act on excised organs. If the drug slows the stream through excised organs, the action must be either on the *endings* (suprarenal) or on the *muscle-fibers* (barium). The distinction between these is not easy. If the endings alone are affected, the drug will not act on every organ, and it will fail to act after apocodein, or after the organ has been excised for some hours. If the effect is on the muscle, it can be obtained in all organs and for many hours after removal from the body, and after apocodein.

Simultaneous Action at Several Points.—The above experiments indicate only the most peripheral structure on which the drug acts. If it affects a peripheral structure and the center simultaneously, a positive distinction is possible only by maintaining a separate artificial circulation through the center. By this means it has been shown that nitrites paralyze the vasoconstrictor mechanism both centrally and peripherally. These experiments, however, are so complicated that they are open to fallacies.

Vasodilation.—The paralysis may be:

1. **Central.**—Stimulation of the peripheral end of the splanchnic nerve raises the blood-pressure; asphyxia, or central stimulation of the sciatic or of the cardiac depressor does not alter the blood-pressure. The paralysis may be *direct* (chloral, chloroform) or *reflex* (depressor stimulation, shock), or the result of extreme asphyxia or anemia. These must be excluded.

2. **Peripheral.**—Stimulation of the splanchnic is ineffective. Paralysis of the *ganglia* (as by nicotin) is excluded by stimulating beyond them. If this is still effective, the action must be on the endings, muscle, or capillaries. If it is on the *endings*, the effect of supra-

renal muscle will be abolished or diminished, but barium will still be effective. Paralysis of the endings is produced by nitrites (probably), apocodein, large doses of ergot, etc. If the *muscle* is paralyzed, even barium will fail to produce a rise.

With arsenic and some other metals there is a fall of pressure of vascular origin, but the vasomotor mechanism responds well to direct or reflex stimulation. These drugs act on the capillary walls. *Capillary paralysis* is also characterized by greater permeability—intravenous injection of salt solution leading readily to muscular edema (Magnus, 1899).

Technical References.—*Lateral pressure in different arteries*, Dawson, 1906, Amer. Jour. Physiol., 15, 244; *Blood-pressure variations in normal dogs*, Hoskins and Wheelan, 1914, Amer. Jour. Physiol., 34, 81; *Percentile Measurement of Vasomotor Reflexes*, Porter, *ibid.*, 33, 373; *Relation Blood-pressure and Respiration*, Th. Lewis, 1908, Jour. Physiol., 37, 213.

TECHNICAL NOTES ON VASOMOTOR NERVES

The **splanchnic nerves** may be stimulated by placing the electrodes, spread fairly wide apart, about the hilus of the suprarenal gland. This may be reached by the same incision as the kidney. To limit the stimu-

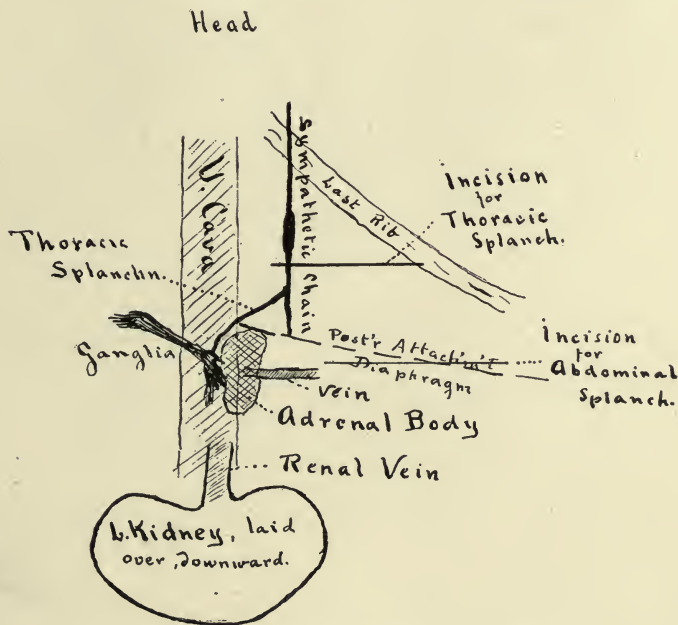


Fig. 60.—Dissection of left splanchnic nerve, dog.

lation strictly to the splanchnics, or to divide these nerves, practice dissections are indispensable. In the rabbit the splanchnic trunks may be found in the thorax, about the tenth dorsal vertebra, on each side of the aorta. The left is the more easily found in the abdomen. It accompanies the aorta until it terminates in the lower celiac ganglion, just above the left suprarenal gland, on the front of the aorta. The right splanchnic is separated from the aorta, in the abdomen, by the vena cava. It terminates in the superior celiac ganglion at the level of the right suprarenal gland, in front of the vein (Figs. 60, 61; Burton-Opiz, 1908, Arch. ges. Physiol., 123, 590).

Technical References.—Tigerstedt, 2,2, 133, Jour. Physiol., 16, 163; Burton-Opitz, Splanchnic and Renal Nerves, Arch. ges. Physiol., 123, 590; Splenic Vessels and Nerves, *ibid.*, 129, 190; Nerves of Portal Vein, Amer. Jour. Physiol., 36, 325; Duodenal Nerves, *ibid.*, 36, 203; Pulmonary Vasomotors, Cloetta and Anderes, Arch. Exp. Path. Pharm., 77, 251.

Destruction of Nerves.—To complete the destruction of nerves, when these accompany vessels, the sheath is painted with concentrated phenol (Bayliss, 1902).

Depressor Nerve.—In cats and dogs this is generally united with the vagosympathetic. It may be stimulated by dividing both vagi and stimulating the cephalic end of the mixed nerve. The results are usually satisfactory in cats, not in dogs.

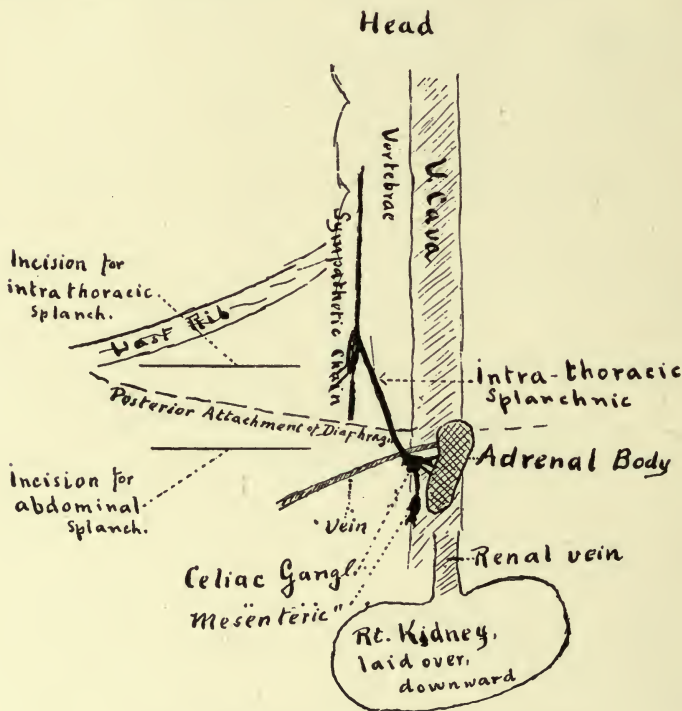


Fig. 61.—Dissection of right splanchnic nerve, dog.

In *rabbits* the depressor runs separately from the vagus and sympathetic. It is the most slender of these nerves, and lies a little to the inner side of the vagus (the largest nerve). It may be identified by the result of stimulation, and by its double origin from the vagus and superior laryngeal. *References:* Tigerstedt, 2,4, 374.

TECHNICAL NOTES ON DESTRUCTION OF THE VASOMOTOR CENTER

The principal vasomotor center is situated in the medulla oblongata extending from 1 or 2 mm. below the corpora quadrigemina downward to within 4 or 5 mm. from the point of the calamus scriptorius, *i. e.*, just above the respiratory center. Subsidiary centers are also situated in the spinal cord.

The vasomotor center may, therefore, be practically excluded by cutting the spinal cord between the calamus and the origin of the vasomotor nerves, which begin about the second dorsal. If the section is made above the fifth cervical nerve, the respiration is also arrested; if made between these regions, the chief vasomotor center is excluded, but the respiration continues. In dogs, a knife thrust through the occipito-atlantoid membrane will divide the medulla just about the lower limit of the vasomotor center.

It should be remembered that there are seven cervical and thirteen dorsal vertebrae, the long spine corresponding to the first dorsal. The first pair of cervical nerves leave through the atlas; the second pair between the atlas and the second vertebra; the third pair between the second and third vertebrae, etc.; the eighth cervical between the last cervical and the first dorsal vertebrae, etc.

Section of the Spinal Cord to Exclude the Vasomotor Center.—The deeply anesthetized and tracheotomized dog is laid on the abdomen, without tying. The neck is rendered prominent by drawing the head over a sand-bag, block, or brick. An incision is made through the skin and muscles, to the spine, from the occiput for a distance of 3 or 4 inches. Artificial respiration is started. The cord is divided between the third and fourth cervical vertebrae. This may be done without removing the vertebrae by pushing a narrow-bladed knife between the articulations. It is more certain, however, to expose the cord. This should be done as quickly as possible, keeping closely to the middle line and to the bone, and the profuse hemorrhage controlled by packing tightly with small pledgets of cotton. The vasomotor centers may be excluded with absolute certainty by destroying the cord, passing a strong brass rod down the spinal canal.

The extent of the section or destruction must always be controlled by sciatic stimulation and by subsequent autopsy.

If it is desired to paralyze the vasomotor center, the spine may be opened from the third cervical vertebra upward, and packed with cotton saturated with 5 per cent. cocaine solution. This may again be rinsed off after a time.

Technical References for Operations on the Cord.—Tigerstedt, 2.4, 338; 3.4, 55.

Technical Notes on Study of Vascular Reactions.—*Inspection of Blood-vessels.*—The vascularity of the organ (rabbit's ear, exposed intestine, kidney, etc.) and the color of the venous blood are noted.

References.—Heinz, 2, 144; Kobert, Intox., 1, 232.

Thermometry.—The temperature of an organ increased with the blood-flow. *References:* Tigerstedt, 2.4, 291.

Oncometry.—See page 169.

Vein Pressure.—The lateral pressure in the inferior cava is measured by connecting the *central* end of the femoral vein with a manometer shaped like the mercury manometer, but filled with water. A little water should be added from time to time to make sure that the vein is not plugged by a clot. A tracing can be obtained by filling the manometer with half-saturated magnesium sulphate and connecting with a Brodie bellows. With some care a cork or hollow aluminum float and aluminum style can be fitted directly to the manometer. A float recorder is described by Hoskins, Gunning, and Berry, 1916, Amer. Jour. Physiol., 41, 517.

References.—Tigerstedt, 2.4, 242, 245.

Peripheral Arterial Pressure (Wolf Method).—The femoral artery is tied, and a cannula connected with the *peripheral* end. Fall in this will indicate dilation of the vessels, and vice versa (Dossin, 1911, Arch. Internat. Pharmacod., 21, 447).

Vein-flow.—In these methods the blood is generally defibrinated and reinjected, or it is rendered non-coagulable by hirudin (see pages 245, 246). An outflow tube is then introduced into the vein, terminally or by a T piece. The outflow is measured or counted; it may also be estimated by the rate of rise of a tambour (see page 168).

Cerebral and Medullary Circulation.—This presents some special problems. *References:* Tigerstedt, 3, 4, 131; *isolation*, Eisenbrey, 1910, Soc. Exp. Biol. Med., 7, 113; E. D. Brown, 1915, Jour. Pharmacol., 6, 603; *excised brains*, see page 171; *Brain volume*, Tigerstedt, 3, 4, 131.

Pulmonary Circulation.—Anderes and Cloetta, 1916, Arch. Exp. Path. Pharm., 79, 291.

Vessel Suture.—*References:* Abderhalden, 5, 815; Carrel, 1912, Surg. Gyn. Obst., 246; Guthrie, 1908, Jour. Amer. Med. Assoc., 51, 1658; *human hair suture*, *ibid.*, 1910, 54, 349; *Vessel-clamp*, G. N. Stewart, 1910, *ibid.*, 55, 647.

Transfusion.—*Measurement with oiled syringe*, Curtis and David, 1910, Jour. Amer. Med. Assoc., 56, 35; *Use of sodium citrate*, R. Weil, 1915, *ibid.*, 64, 425. (About 1 c.c. of 10 per cent. per 10 c.c. of drawn blood; its injection causes no disturbance and does not change the coagulation time of the circulating blood.) *Methods and apparatus*, Jour. Amer. Med. Assoc., 1916, 66, 1923.

Plasmapheresis (Plasma removal with return of corpuscles).—Withdrawal of blood with re-injection of the corpuscles suspended in 0.6 per cent. NaCl. Much larger quantities can be withdrawn than in simple bleeding (Abel, Rowntree, and Turner, 1914, Jour. Pharmacol., 5, 611).

Compression of Arteries.—Metal band, Matas and Allen, 1911, Jour. Amer. Med. Assoc., 56, 233.

EXERCISE I.—(GROUP I) NITRITE AND EPINEPHRIN; RELATION OF RESPONSE TO LEVEL OF BLOOD-PRESSURE

Distribution of Work.—Student B—Director and Reporter; calculates doses; takes notes and prepares report.

Student E—Chief Operator.

Student F—Assistant Operator; weighs animal; gives injections.

Student A—Anesthetist; artificial respiration and resuscitation if necessary; cleaning.

Student C—Pulse; blood-pressure tracings (pages 242–246).

Student D—Respiratory tracing (page 239).

Observations.—Heart-rate; blood-pressure tracing; respiratory tracing (Stephen Hale experiment).

Apparatus.—*Stephen Hale manometer:* glass tubing 4 mm. diameter, 10 to 14 feet high, suspended vertically, with rubber connection to carotid. The interior of the tube and connection should be well oiled, or leech extract may be used. Mercury manometer with screw-clamp on connection for blood-pressure tracing. Tracheal tube and tambour for respiration. Two injection burets. Induction coil.

Animal.—Morphinized dog or cat with M. A. U. anesthetic.

Operation.—Weigh animal. Etherize and tie to board. Place cannulae into carotid, trachea, and femoral veins with burets (one for epinephrin, 1 : 1000; the other for nitroglycerin, 1 : 1000).

Experiment 1. Epinephrin.—Open the carotid artery and let the blood rise in the tube. When it has reached its maximum, measure the height of the column in the systole and diastole.

Inject into vein Epinephrin, 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg. Measure height of column.

Experiment 2. Amyl Nitrite.—When pressure has returned to normal, let the animal inhale the nitrite. Measure height of column.

Operation.—Disconnect the manometer tube (and wash it before the blood clots). Dissect the left splanchnic nerve and place on electrodes. Connect carotid with mercury manometer and tighten screw-clamp till

excursions are quite small. Connect trachea for respiratory tracing. Start tracings.

Experiment 3. Splanchnic Stimulation.—While taking tracings stimulate splanchnic nerve with moderate shocks until the blood-pressure has reached a maximum. Let pressure return to normal.

Experiment 4. Nitroglycerin.—Inject into vein Nitroglycerin 0.5 mg. (0.5 c.c. of 1 : 1000) per kg. When the pressure has reached a minimum, proceed to Experiment 5.

Nitroglycerin and nitrites produce considerable fall of blood-pressure and increase of vein-pressure and oncometer (vasomotor paralysis); and some quickening of the pulse (vagus depression). Respiration usually increases. The effects pass off rapidly.

If the vagus was depressed before the nitrite was given—as denoted by fast pulse—there may not be any further quickening.

Experiment 5. Splanchnic Stimulation During Nitroglycerin Fall.—When the blood-pressure has reached its minimum under nitroglycerin again stimulate the splanchnic until pressure ceases to rise. Let conditions return to normal.

Experiment 6. Compression of Aorta.—Clamp aorta where it emerges from diaphragm.

Experiment 7. Nitroglycerin During Compression of Aorta.—Leave clamp on aorta. When pressure ceases to rise inject nitroglycerin as in Experiment 4.

Experiment 8. Compression of Aorta During Nitroglycerin Fall.—Inject nitroglycerin until pressure has fallen to the minimum of Experiment 4. Then clamp aorta until pressure ceases to rise. Release aorta.

Experiment 9. Epinephrin.—When conditions have returned to normal, inject into vein Epinephrin, 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.: rise in blood-pressure and fall in vein-pressure and oncometer (peripheral vasoconstriction); slower pulse (vagus stimulation) and stronger heart (stimulation cardiac muscle); respiration usually increased (higher blood-pressure?). Cardiac slowing often precedes the vasoconstriction. Note that the effects disappear rapidly.

Experiment 10. Epinephrin During Nitroglycerin.—When conditions have returned to normal, inject nitroglycerin as in Experiment 4. When the pressure has fallen to the minimum, inject epinephrin as in Experiment 9.

Experiment 11. Nitroglycerin During Epinephrin.—Inject epinephrin very slowly, adjusting stop-cock so that a uniform, moderate rise of pressure of 30 to 50 mm. is maintained. When this condition is reached, and while the epinephrin is still running in, inject with a hypodermic syringe into the vein connection nitroglycerin, as in Experiment 4. When pressure has fallen to minimum, discontinue the epinephrin and let conditions return to normal.

Experiment 12. Nitroglycerin During Hemorrhage.—Insert cannula into femoral artery and withdraw blood until pressure has fallen by 25 to 40 mm. Inject nitroglycerin as in Experiment 4.

Experiment 13. Strophanthus.—Clean epinephrin buret and through it inject strophanthus, 1 mg. ($\frac{1}{10}$ c.c. of 1 : 100) per kg.

Experiment 14. Nitroglycerin During Strophanthus.—When pressure ceases to rise, inject nitroglycerin as in Experiment 4.

QUESTIONS

Describe the effects of:

- (a) Epinephrin (Experiments 1 and 9).
- (b) Amyl nitrite and nitroglycerin (Experiments 2 and 4).
- (c) Splanchnic stimulation (Experiment 3).
- (d) Compression of aorta (Experiment 6).
- (e) Hemorrhage (Experiment 12).
- (f) Strophanthus (Experiment 13).

How efficiently does nitroglycerin counteract rise of pressure produced by:

- (g) Compression of aorta? (Experiment 7.)
 - (h) Epinephrin? (Experiment 11.)
 - (i) Strophanthus? (Experiment 12.)
- (Compare these with the millimeter fall and with the level of pressure reached in Experiment 4.)
- (k) Where would the action of the nitroglycerin be located? (Epinephrin acts on the myoneural junction; strophanthus on the arterial muscle).
 - (l) Which vascular area is mainly affected by the nitroglycerin. (Compare Experiments 7 and 4).
 - (m) How does hemorrhage affect the nitroglycerin fall? (Compare Experiments 12 and 4.) Explain.
 - (n) How does nitroglycerin affect the response to splanchnic stimulation? (Compare Experiments 3 and 5.) Explain bearing on location of nitroglycerin action.
 - (o) How efficiently does epinephrin counteract the nitroglycerin fall? (Compare Experiments 9 and 10.) Explain bearing on location of nitroglycerin action.
 - (p) Summarize evidence as to location of nitroglycerin action.
 - (q) State some possible therapeutic applications of nitroglycerin.

OPTIONAL VASOMOTOR EXPERIMENTS

Experiment 15. Doses of Various Vasomotor Drugs Not Used in the Regular Experiments.—Aconite: 15 mg. per kg., ineffective; 100 mg. per kg., fatal.

Berberin: 1 mg. per kg.

Camphor: 5 mg. per kg.

Cyanid Potassium: 1 mg. per kg., blood-pressure rise.

Digitalis: 50 mg. per kg., rapid therapeutic action; 100 mg. per kg., toxic.

Ergotoxin: 0.25 mg. per kg.

Ether: $\frac{1}{4}$ to 2 c.c. of sat. sol. per kg.

Hydrastin: 5 mg. per kg.

Lactic Acid: 2 c.c. of 0.6 per cent. per kg.

Sparteïn: 5 mg. per kg.

Strychnin: 0.5 mg. per kg., tetanic; 1 mg. or over per kg., depression of vasomotor center.

Experiment 16. Position on Blood-pressure.—Tigerstedt, 2.4, 302.

Experiment 17. Cerebral Compression.—H. Cushing, 1902, *Grenzg. Med. Chir.*, 9, 793; Eyster, Burrows, and Essick, *Jour. Exp. Med.*, 11, 489; Tigerstedt, 2.4, 288.

Experiment 18. Depressor Stimulation with Vasomotor Drugs.—Sollmann and Pilcher, 1912, *Amer. Jour. Physiol.*, 30, 369.

Experiment 19. Thyroid Sensitization of Depressor and Epinephrin.—Asher and Flack, 1911, *Zs. Biol.*, 55, 83 (one "tablet" in 10 c.c. of dilute alkali; filter; 2 c.c. of filtrate per kg., vein).

Experiment 20. Heating of Carotid Blood.—Stewart, 297.

Experiment 21. Clamping of Carotid Arteries; Traction on Cephalic End of Carotid.—Sollmann and Brown, 1912, *Amer. Jour. Physiol.*, 30, 88.

EXERCISE II.—(GROUP II) PERIPHERAL VASOMOTOR DRUGS ON BLOOD-PRESSURE AND KIDNEY VOLUME, WITH INSPECTION OF INTESTINAL VESSELS

Distribution of Work.—Student B—Director and Reporter; calculates doses; takes notes and prepares report.

Student E—Chief Operator.

Student F—Assistant Operator; weighs animal; gives injections.

Student A—Anesthetist; artificial respiration and resuscitation if necessary; cleaning.

Student C—Pulse; blood-pressure tracings.

Student D—Kidney volume (page 169) and watch color of intestines.

Observations.—Heart-rate; blood-pressure tracing; kidney oncometer record or tracing; color of intestines (anemia, congestion, etc.). If the oncometer is merely read from the manometer, the readings should be recorded at the proper place on the tracing.

Apparatus.—Damped mercury manometer for blood-pressure tracing; tracheal cannula; kidney oncometer with water-manometer (and recording device, if desired). Injection buret; induction coil.

Animal.—Morphinized dog, or cat with M. A. U. anesthetic.

Operation.—Weigh the animal. Etherize and tie on board. Place cannulae into carotid, trachea, and femoral vein. Open abdomen and arrange kidney in oncometer. Draw out a loop of intestine for observation (cover with warm towel). Connect carotid for tracing.

Injections.—Make all injections into vein; let conditions return as near as possible to normal between injections.

Experiment 1. Strychnin (Therapeutic Dose).—Inject Strychnin, 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg. Generally no noticeable result (this would correspond to about $\frac{1}{40}$ grain for a man).

Experiment 2. Amyl Nitrite.—Administer by inhalation (see Exercise I, Experiments 2 and 4, page 271).

Experiment 3. Epinephrin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg. (see Exercise I, Experiment 9, page 271).

Experiment 4. Pituitary.—Inject Pituitary Solution, 0.1 c.c. per kg.: moderate but rather prolonged rise of pressure, often preceded by short fall; intestinal vessels contract, kidney may dilate (peripheral constriction of vessels; cardiac depression). Intestinal movements increased.

Experiment 5. Ergot.—Inject 250 mg. (1 c.c. of 25 per cent.) per kg. Effects variable: generally a moderate rise, which may be preceded by a temporary fall.

During the fall the heart is weakened and quickened, and the oncometer is diminished. The fall is therefore due to weakening of the heart. During the rise the heart is strengthened; the oncometer may increase or remain stationary. The rise is consequently due to strengthening of the heart, with some vasoconstriction.

During the fall the heart is quickened and the respiration increased. This is due to the low blood-pressure. The cardiac effects can be reproduced on excised hearts and are therefore direct actions.

The fall of pressure is not seen when the ergot is injected subcutaneously or into the muscles.

Experiment 6. Tyramin.—Inject 2 mg. (2 c.c. of 1 : 1000) per kg.: rise of blood-pressure by peripheral vasomotor (Tyramin, Histamin and Cholin, together with Ergotoxin are active constituents of Ergot).

Experiment 7. Histamin.—Inject 0.01 mg. ($\frac{1}{10}$ c.c. of 1 : 10,000) per kg.: fall of blood-pressure.

Experiment 8. Cholin.—Inject 2 mg. (2 c.c. of 1 : 1000) per kg.: rise of blood-pressure.

(Optional) This fall does not occur after the intravenous injection of atropin—1 mg. for cats.

Experiment 9. Cotarnin.—Inject 5 mg. ($\frac{1}{2}$ c.c. of 1 : 100) per kg.: fall of blood-pressure. (This substance has been tried as a hemostatic.)

Experiment 10. Hydrastis.—Inject 20 mg. (1 c.c. of 2 per cent., filtered) per kg. Short fall of pressure, followed by persistent rise. Both phenomena are in part cardiac, in part vascular. The oncometer results are therefore variable.

Experiment 11. Hydrastinin.—Inject 5 mg. ($\frac{1}{2}$ c.c. of 1 : 100) per kg. Rise of pressure, mainly cardiac. (Hydrastinin is an artificial derivative of Hydrastin, a hydrastic alkaloid; it has been suggested as a circulatory stimulant, but has not found much application.)

Experiment 12. Nicotin.—Expose the vagus and find the smallest stimulus which just stops the heart. Inject Nicotin, 0.1 mg. ($\frac{1}{10}$ c.c. of 1 : 1000) per kg. The peristalsis is greatly increased. The respiration is also increased and the animal may become convulsive. When the heart has become quickened, note that stimulation of the vagus does not stop the heart (depression of the vagus ganglion cells). Very strong stimulation may cause some slowing if the paralysis is incomplete.

Experiment 13. (Optional) Nicotin on Ganglia and Nerve-fibers.—Expose the superior cervical ganglion of an anesthetized rabbit. Stimulation causes constriction of the ear vessels and dilation of the pupil. Paint 1 per cent. nicotin on the nerve below the ganglion. A stimulus applied central to this point is still effective, showing that the nerve-fibers are not paralyzed by the poison. Paint the nicotin on the ganglion. Stimulation of the nerve is now ineffective, showing paralysis of the ganglion.

QUESTIONS

(a) Tabulate the effects of the drugs on pulse-rate, blood-pressure, kidney volume, and intestinal vessels.

(b) The organ volume or congestion varies in the same direction as the blood-pressure if a change is cardiac, and inversely if it is vascular. On this basis, state for each of these drugs whether the blood-pressure change is cardiac or vascular.

EXERCISE III.—(GROUP III) PERIPHERAL AND CENTRAL VASOMOTOR DRUGS ON BLOOD-PRESSURE, INTESTINAL VOLUME, AND RESPIRATION: TREATMENT OF PEPTONE SHOCK

Distribution of Work.—Student B—Director and Reporter; calculates doses; takes notes and prepares report.

Student E—Chief Operator.

Student F—Assistant Operator; weighs animal; gives injections.

Student A—Anesthetist; artificial respiration and resuscitation if necessary; cleaning.

Student C—Pulse; blood-pressure tracings (pages 242–246).

Student D—Respiratory tracing (page 239) and intestinal oncometer (page 169).

Observations.—Heart-rate; blood-pressure tracing; respiratory tracing; intestinal oncometer, record or tracing; color of intestines (anemia, conges-

tion, etc.). If the oncometer is merely read from the manometer, the readings should be recorded at the right place on the tracing.

Apparatus.—Damped mercury manometer for blood-pressure tracing. Tracheal cannula. Intestinal oncometer with water-manometer (and recording device if desired). Injection buret.

Animal.—Morphinized dog, or cat with M. A. U. anesthetic.

Operation.—Weigh the animal. Etherize and tie on board. Place cannulae into carotid, trachea, and femoral vein. Open abdomen and arrange intestinal loop in oncometer. Draw out a loop of intestine for observation (cover with warm towel). Connect carotid for tracing.

Injections.—Make all injections into vein; let conditions return as near as possible to normal between injections.

Experiment 1. Strychnin (Therapeutic Dose).—Inject Strychnin, 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.: generally no noticeable result (this would correspond to about $\frac{1}{20}$ grain for a man).

Experiment 2. Sodium Nitrite.—Inject 5 mg. ($\frac{1}{20}$ c.c. of 10 per cent.) per kg. (See Exercise I, Experiment 4.)

Experiment 3. Epinephrin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg. (See Exercise I, Experiment 9.)

Experiment 4. Alcohol.—Inject 1 c.c. (4 c.c. of 25 per cent.) per kg.

Experiment 5. Sodium Diethyl Barbiturate (Veronal).—Inject 0.2 gm. (2 c.c. of 10 per cent.) per kg. (Jacobj and Roemer, 1911, Arch. exp. Path. Pharm., 66, 241).

Experiment 6. Peptone Shock.—Inject Witte's Peptone slowly, 0.2 to 0.5 gm. (2–5 c.c. of 10 per cent.) per kg., until pressure remains below 40 mm. The oncometer also falls, but the large splanchnic veins appear congested (probably loss of tone of splanchnic vessels). The condition is probably similar to ordinary "shock."

Treatment of Shock.—Note efficiency or failure of the following procedures:

Experiment 7. Ammonia Inhalation.—Blow Ammonia vapor into nose: little or no effect.

Experiment 8. Saline Infusion.—Inject warm Saline, 5 to 25 c.c. per kg.: no improvement; on the contrary, increase of splanchnic congestion.

Experiment 9. Epinephrin.—Repeat Experiment 3: diminished response.

Experiment 10. Strophanthus.—Inject 1 mg. (0.1 c.c. of 1 : 100) per kg.: fair response. When pressure ceases to rise.

Experiment 11. Epinephrin During Strophanthus.—Make continuous slow injection of Epinephrin: the blood-pressure can be maintained at an effective level (Pearce and Eisenbrey, 1910, Arch. Int. Med., 6, 218).

QUESTIONS

(a) Tabulate the effects of the drugs of Experiments 1 to 6 on respiration, pulse-rate, blood-pressure, intestinal volume, and intestinal vessels.

(b) The organ volume or congestion varies in the same direction as the blood-pressure if a change is cardiac, and inversely if it is vascular. On this basis, state for each of these drugs whether the blood-pressure change is cardiac or vascular.

(c) What relations have the respiratory changes to the blood-pressure?

(d) Describe the results of the treatment of "shock" and discuss the efficiency of the measures.

TECHNICAL NOTES

Traumatic (Surgical) Shock.—The intestines of the anesthetized animal are exposed and severely manipulated.

Toxic Shock (Diphtheria Toxin).—Dosage, etc., H. Meyer, Arch. Exp. Path., 60.

EXERCISE IV.—(GROUP IV) PERIPHERAL AND CENTRAL VASOMOTOR DRUGS ON BLOOD-PRESSURE AND HEART (CARDIOPLETHYSMOGRAPH)

Distribution of Work.—Student E—Director and Chief Operator.

Student F—Assistant Operator; weighs animal; gives injections.

Student A—Anesthetist; artificial respiration and resuscitation if necessary; cleaning.

Student B—Reporter; calculates doses; takes notes and prepares report.

Student C—Pulse; blood-pressure tracings.

Student D—Cardiac tracing.

Observations.—Heart-rate; blood-pressure tracing; cardioplethysmogram.

Apparatus.—Damped mercury manometer for blood-pressure tracing. Tracheal cannula. Cardioplethysmograph and insufflation (pages 259, 260). Injection buret. Induction coil.

Animal.—Morphinized dog.

Operation.—As in Chapter XLII, page 258. Also place cannula into femoral artery.

Injections.—All intravenous. Let conditions return as near as possible to normal between injections.

Experiment 1. Strychnin (Therapeutic Dose).—Inject Strychnin, 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.: generally no noticeable result (this would correspond to about $\frac{1}{20}$ grain for a man).

Experiment 2. Nitroglycerin.—Inject 0.5 mg. ($\frac{1}{20}$ c.c. of 1 : 100) per kg. (See Exercise I, Experiment 4, page 271.)

Experiment 3. Hemorrhage.—Draw blood from femoral artery so as to produce the same blood-pressure changes as with the nitroglycerin. Compare results.

Experiment 4. Epinephrin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg. (See Exercise I, Experiment 9, page 271.)

Experiment 5. Aortic Compression.—Compress aorta at diaphragm, so as to produce the same blood-pressure changes as with the epinephrin. Compare the results.

Experiment 6. Pituitary.—Inject Pituitary Solution, 0.1 c.c. per kg. (See Exercise II, Experiment 4, page 273.)

Experiment 7. Phenol (Toxic Dose).—Inject 30 mg. (3 c.c. of 1 per cent.) per kg.: collapse. Pressure falls (vasomotor and cardiac paralysis, beats fast and small (cardiac depression); respiration lessened (depression of center); convulsive (stimulation spinal cord).

Experiment 8. Chloral.—Inject 0.5 gm. (5 c.c. of 10 per cent.) per kg.: fall of pressure, vasomotor and cardiac.

Experiment 9. Strophanthus.—Inject 1 mg. (0.1 c.c. of 1 : 100) per kg.: rise of pressure and cardiac changes similar to digitalis, but more prompt. (See Chapter XLV, Exercise III, Experiment 4, page 286.)

Experiment 10. Arsenic.—Dissect left splanchnic and note effect of its stimulation on blood-pressure. Also note appearance of intestine. Inject Sodium Arsenate, 50 mg. (1 c.c. of 5 per cent.) per kg.: the blood-pressure

falls and the intestines appear congested. Stimulate the splanchnic; if the action has not gone too far, there is a good response, showing that the arterioles and their innervation are still effective. Compress the aorta: the pressure rises, showing that the efficiency of the heart is maintained. The paralysis is in the capillaries. Continue the observation of the animal, and when the pressure falls further repeat the splanchnic and aortic tests. The response will decrease, as in all conditions of low blood-pressure.

QUESTIONS

(a) Tabulate the effects on the blood-pressure and heart-rate, excursions, systolic and diastolic tone.

(b) In how far may the cardiac effects of each drug be the indirect result of the blood-pressure changes? (Compare with Experiments 3 and 5.)

(c) Discuss the evidence for the location of the arsenic fall.

EXERCISE V.—(GROUP V) REACTIONS OF THE VASOMOTOR CENTER (PERFUSION METHOD)

Outline of Method.—(Sollman and Pilcher, 1910, Amer. Jour. Physiol., 26, 233.)

The vessels of the spleen, kidney, etc., are ligated and perfused, leaving the nerves uninjured. The circulation of the organ is thus completely severed from that of the animal and can only be influenced through the vasomotor center. The vein-flow from the organ being recorded, a slowing must correspond to central vasoconstriction, quickening of the flow to central vasodilation.

Distribution of Work.—Student B—Director and Reporter; calculates doses; takes notes and prepares report.

Student E—Chief Operator.

Student F—Assistant Operator; weighs animal; gives injections.

Student A—Anesthetist; artificial respiration and resuscitation if necessary; cleaning.

Student C—Pulse; blood-pressure tracings.

Student D—Vein outflow.

Observations.—Heart-rate; blood-pressure tracing; outflow tracing.

Apparatus.—Bellows for artificial respiration. Damped mercury manometer. Tracheal cannula. Two-liter Mariotte bottle, suspended 4 feet above animal, connected with Wolff bottle, resting in water-bath at 40° C. This in turn is to be connected with the artery, the whole arrangement being filled with Locke's fluid. Outflow tube, connected with the vein, and delivering into a "dipping bucket," connected with electric time-marker on drum. Curare, $\frac{1}{2}$ per cent.

Animal.—Morphinized dog.

Operation.—Weigh animal. Etherize. Tie on board. Place cannulae in carotid, trachea, and femoral vein. Expose spleen (or kidney) through small incision. The largest artery and its accompanying vein are reserved. All the remaining vessels and tissues are tied off in two masses by strong ligatures. The reserved artery and vein are then cleaned with blunt needle, avoiding injury to the nerves.

The perfusion cannula is next tied into the artery (again avoiding the nerves) and the perfusion is started. When the spleen has been somewhat flushed, the outflow cannula is placed in the vein and connected with the

dipping bucket. Both cannulæ point toward the spleen. They are fixed in position. The tracings are now started. Should the animal struggle, artificial respiration is started and curare injected ($\frac{2}{3}$ c.c. of $\frac{1}{2}$ per cent. per kg.).

Injections.—These are all made into the femoral vein. Let conditions return to normal between the experiments.

Experiments.—Determine the effects of the following procedures:

1. Asphyxia.
2. Hemorrhage, slight and severe (defibrinate the blood).
3. Reinjection of defibrinated blood.
4. Asphyxia.
5. Nitroglycerin, 0.5 mg. ($\frac{1}{20}$ c.c. of 1 : 100) per kg.
6. Epinephrin, 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.
7. Strychnin (therapeutic dose), 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.
8. Chloroform inhalation.
9. Caffein, 10 mg. (1 c.c. of 1 per cent.) per kg.
10. Cevadin, 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.
11. Atropin, 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.
12. Cevadin, as in 10.
13. Strophanthus, 1 mg. ($\frac{1}{10}$ c.c. of 1 per cent.) per kg.
14. Strophanthus, 5 mg. (0.5 c.c. of 1 per cent.) per kg.

QUESTIONS

- (a) Tabulate the effects on heart-rate and vasomotor center.
- (b) State which of the drugs owe their circulatory effects mainly to the vasomotor center and which do not.
- (c) Which drugs owe their action on the vasomotor center mainly to asphyxia?
- (d) What are the effects of low blood-pressure? How are they explained?

TECHNICAL REFERENCE

Outflow Recorder.—A simple syphon recorder is described by Gunn, 1913, Proc. Physiol., Soc., Oct.

EXERCISE VI.—(OPTIONAL) CIRCULATION TIME

The efficiency of the circulation depends mainly on the velocity of the blood-stream, the "mass-movement" of the blood. This may be measured in several ways, the methylene-blue method of G. N. Stewart being the simplest. A 2 per cent. solution, about $\frac{1}{4}$ c.c. per kg., is injected into the jugular vein. A stop-watch is used to time the interval between its arrival at a given artery (for instance, in a loop of intestine) and its passage from here into the corresponding vein. The recognition of the color change is greatly facilitated by the use of transmitted light. Observations are made before and during the actions of the drugs.

The following may be tried (intravenous injections):

- (1) Vagus stimulation.
- (2) Epinephrin, 0.05 mg. per kg.
- (3) Nitroglycerin, 0.5 mg. per kg.
- (4) Alcohol, 1 c.c. per kg.
- (5) Caffein, 10 and 50 mg. per kg.
- (6) Strophanthus, 1 and 3 mg. per kg.

TECHNICAL REFERENCE

Tigerstedt, 2.4, 304.

EXERCISE VII.—(OPTIONAL) BLOOD-PRESSURE ASSAY OF SUPRARENAL PREPARATIONS

One of the most reliable methods for estimating the strength of a suprarenal preparation consists in determining the dose required to produce a definite, moderate (30 to 60 mm.) rise of blood-pressure, and comparing this with a known preparation (about 1 c.c. of 1 : 100,000 epinephrin per dog). Details are given in the U. S. P. Dried suprarenal gland should contain at least 1 per cent. of epinephrin.

The most uniform results are obtained by pithing the brain and spinal cord (through the orbit), dividing both vagi and sympathetics, and giving artificial respiration (Elliott, 1914, *Jour. Physiol.*, 44, 374; R. L. Levy, 1916, *Amer. Jour. Physiol.*, 41, 495).

TECHNICAL REFERENCES

U. S. P. IX; *Jour. Amer. Med. Assoc.*, 57, 1149, 1911; *Jour. Amer. Pharm. Assoc.*, 1, 1305, 1912; Pittenger, 52.

Other Methods.—See page 167.

CHAPTER XLIV

CHANGES IN HEART-RATE, ETC.

(REPORTERS: A MEMBERS OF EACH GROUP)

Introduction.—The influence of the heart-rate on the filling and output of the cardiac chambers is of great therapeutic importance.

Influence of Heart-rate on Output and Blood-pressure.—The minute-output of the heart, and with it the blood-pressure, increases with the rate: rapidly up to about 120 beats per minute; relatively less between 120 and 210 beats; and declines above 210 beats.

Y. Henderson, 1909 (*Amer. Jour. Physiol.*, 23, 345), finds that the output of blood with each heart-beat under normal conditions of the circulation depends mainly on the diastolic filling, and that this varies inversely to the heart-rate. The minute-volume varies with the heart-rate. The output per beat (the difference between diastolic and systolic volume)

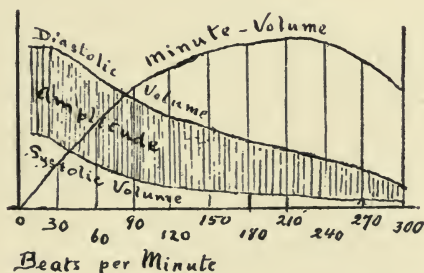


Fig. 62.—Relation of heart-rate to amplitude (output per beat) and minute-volume under normal circulatory conditions (after Henderson). The amplitude (shaded) corresponds to the difference between the diastolic and systolic volumes.

does not vary much with pulse-rates to about 80 per minute—to this point the minute-volume, therefore, increases with the rate. Above 80 the amplitude decreases progressively until, above 240 beats per minute, it has fallen so much that the minute-output is also decreased (Fig. 62).

Control of the Heart-rate.—The rate of the heart is controlled by inhibitory impulses of the vagus, by the augmentory impulses of the accelerators, and by the state of the cardiac muscle. Any of these may be affected by drugs, the nervous structures both peripherally and centrally, directly and reflexly. The methods of analysis were discussed in Chapter XXXVI, page 185.

It may be recalled that *slowing* by central or reflex *stimulation of the vagus* will not occur if the vagi have been previously divided. Peripheral vagus stimulation would occur after vagotomy, but would be abolished by atropin.

Quickening by depression or inhibition of the *vagus* center will be removed by stimulation of the vagus nerves. If the vagus endings have been paralyzed, stimulation of the vagus trunk will be ineffective. Stimulation of the *accelerator center* may be excluded by section of these nerves.

Changes of blood-pressure influence the heart-rate mainly through the vagus center, so that rise of pressure generally slows; and fall of pressure quickens, the rate. The blood-pressure factor may be excluded, either by comparing the effect with equal changes of pressure produced by hemorrhage or aortic compression (Exercise V), or by keeping the blood-pressure constant by a *compensating device* (Bayliss, 1908, Jour. Physiol., 37, 272; Jackson, 1913, Jour. Pharmacol., 4, 291).

Dissection of Vagus Nerves.—This was described in Chapter XLI.

Dissection of Accelerator Nerves and Stellate Ganglia.—(The dissection should be practised in advance.) The dog is tied on its back, front legs drawn down. The operator stands at the head. Median incision in neck extending an inch over the manubrium. Cross incision on either side between first and second rib. Isolate sternomastoid insertions and divide. Isolate pectoral insertions and divide. Clean median vein and divide between double ligature. Pull external jugular outward. Divide internal jugular between double ligature. Follow carotids (pull in) and vagi to subclavian. Clean subclavian and first two branches (vertebral and costocervical) from fat. Start oxygen and curare and resect one or two ribs. Now isolate the nerves, beginning at left side. The branches of the inferior cervical ganglion run, from out to in:

Small fibers.

Annulus behind.

Annulus before.

Vagus stem.

Accelerator.

Anastomosis with vagus.

Anastomosis with inferior laryngeal.

Follow the annulus to the stellate ganglion, pulling the subclavian up and aboral. The ganglion is rather outward (inward and below from origin of vertebral artery).

Technical Reference.—Tigerstedt, 2.4, 353; Anderson, 1904, Jour. Physiol., 31, 2.

EXERCISE I.—(GROUP I) HEART-RATE ON BLOOD-PRESSURE AND CARDIAC EXCURSIONS (CARDIOMYOGRAPH)

Distribution of Work.—Student A—Director and Reporter; calculates doses; takes notes and prepares report.

Student D—Chief Operator.

Student E—Assistant Operator; weighs animal; gives injections.

Student F—Anesthetist; artificial respiration; cleaning.

Student B—Pulse; blood-pressure tracings.

Student C—Cardiograph tracings.

Observations.—Heart-rate; blood-pressure tracing; myocardiograph tracing from ventricle and, if possible, from auricle; inspection of heart.

Fast tracings should be taken at the critical points.

Apparatus.—Damped mercury manometer for blood-pressure tracing. Insufflation anesthesia (Chapter XLII, page 258). Myocardiograph (Cushny, 1910, Heart, 2, 1). Induction coil.

Animal.—Morphinized dog or M. A. U.¹ cat.

Operation.—As in Chapter XLII, Exercise VI, page 263, but adjusting the cardiograph instead of plethysmograph.

Injections.—All into femoral vein. Let conditions return to normal between the experiments.

Experiment 1. Weak Vagus Stimulation.

Experiment 2. Maximal Vagus Stimulation.

Experiment 3. Cevadin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.: marked slowing or cardiac arrest, with prompt escape (veratrum viride acts similarly).

Experiment 4. Section of Vagi.—Divide both vagi

Experiment 5. Cevadin After Division of the Vagi.—Repeat Experiment 3; no effect on heart-rate.

Experiment 6. Strophanthus.—Inject 1 mg. ($\frac{1}{10}$ c.c. of 1 per cent.) per kg. Repeat every ten minutes till death. (See Chapter XLV, Exercise II, Experiment 4.)

Questions.—(a) Describe the effects of slowing the heart on the blood-pressure and excursions, diastolic volume, and systolic volume of heart (Experiments 1, 2, and 3).

(b) Describe the effects of tachycardia, ditto (Experiment 4).

(c) Describe the effects of cevadin (Experiment 3).

(d) On what structures does it act? (Compare Experiments 3 and 4.)

(e) Describe the effects of strophanthus.

(f) How does the heart behave differently under strophanthus and under vagus stimulation?

(Optional) **Strophanthus on Febrile Heart.**—Induce fever (page 224) or heat the carotid blood (page 272), and try the effect of strophanthus.

EXERCISE II.—(GROUP II) HEART-RATE ON BLOOD-PRESSURE AND CARDIAC EXCURSIONS (CARDIOPLETHYSMOGRAPH)

Read remarks under Introduction, page 279.

Distribution of Work.—As in Exercise I, page 280.

Observations.—Heart-rate; blood-pressure tracings; cardioplethysmogram; inspection of heart.

Apparatus and Operations.—As in Chapter XLII, Exercise VI, page 263. Induction coil.

Animal.—Morphinized dog or M. A. U. cat.

Injections.—All into femoral vein. Let conditions return to normal between the experiments.

Experiment 1. Weak Vagus Stimulation.

Experiment 2. Maximal Vagus Stimulation.

Experiment 3. Cevadin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.: marked slowing or cardiac arrest, with prompt escape (veratrum viride acts similarly).

Experiment 4. Spartein.—Inject 5 mg. ($\frac{1}{2}$ c.c. of 1 per cent.) per kg.: brief rise of pressure; more lasting slowing of rate; weakening of cardiac contractions.

¹ M. A. U. stands for morphin-atropin-urethane anesthesia, page 248.

Experiment 5. Pilocarpin.—Inject 1 mg. ($\frac{1}{10}$ c.c. of 1 : 100) per kg.: heart first slowed; later it may be quickened (peripheral vagus stimulation and depression). Cats may show pulmonary edema.

Experiment 6. Digitalis.—Inject 50 mg. (1 c.c. of 5 per cent.) per kg., as in Chapter XLV, Exercise III, Experiment 4.

QUESTIONS

(a) Describe the effects of slowing the heart on the blood-pressure and excursions, diastolic volume, and systolic volume of heart (Experiments 1, 2, and 3).

(b) Describe the effects of cevadin, spartein, pilocarpin, and digitalis.

(c) Discuss whether their cardiac effects are referable simply to the change of heart-rate.

EXERCISE III.—(GROUP III) HEART-RATE, ETC., ON BLOOD-PRESSURE AND ORGAN VOLUME

Fast tracings may be taken at the critical points.

Distribution of the Work.—Student A—Director and Reporter; calculates doses; takes notes and prepares report.

Student D—Chief Operator.

Student E—Assistant Operator; weighs animal; gives injections.

Student F—Anesthetist; artificial respiration; cleaning.

Student B—Pulse; blood-pressure tracings.

Student C—Kidney oncometer; inspection of intestinal vessels.

Observations.—Heart-rate; blood-pressure tracing; kidney oncometer; intestinal vessels.

Apparatus.—Damped mercury manometer for blood-pressure tracing. Tracheal cannula. Oncometer. Induction coil.

Animal.—Morphinized dog or M. A. U. cat.

Operation.—Weigh animal. Etherize. Tie on board. Place cannula in carotid, trachea, and femoral vein. Expose kidney and arrange in oncometer. Draw out loop of intestine for inspection. Start tracings.

Injections.—All into vein.

Experiment 1. Weak Vagus Stimulation.

Experiment 2. Maximal Vagus Stimulation.

Experiment 3. Cevadin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.: marked slowing or cardiac arrest, with prompt escape (veratrum viride acts similarly).

Experiment 4. Dog's Urine.¹—Inject about 3 c.c.: large fall of blood-pressure.

Experiment 5. Ouabain (Crystallized Strophanthus).—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg. Repeat every ten minutes till death. (See Chapter XLV, Exercise III, Experiment 4.)

QUESTIONS

(a) Describe the effects of cardiac slowing on the blood-pressure, kidney volume, and large intestinal veins (Experiments 1 and 2). Explain.

(b) Describe the effects of cevadin, urine, and ouabain on these functions and on the heart-rate.

(c) Explain their actions.

¹ *Urine Depressor Substances.*—Pearce and Eisenberg, 1910, Amer. Jour. Physiol., 26, 26.

Fecal Depressor Substances.—Wallace and Sturtevant, 1914, Soc. Exp. Biol. Med., 11, 114.

EXERCISE IV.—(GROUP IV) HEART-RATE ON BLOOD-PRESSURE AND URINE FLOW

The flow of urine depends largely on the flow of blood through the kidneys and may, therefore, be influenced by the circulation.

Distribution of Work.—Student A—Director and Reporter; calculates doses; takes notes and prepares report.

Student D—Chief Operator.

Student E—Assistant Operator; weighs animal; gives injections.

Student F—Anesthetist; artificial respiration; cleaning.

Student B—Pulse; blood-pressure tracings.

Student C—Urine flow; inspection of kidney.

Observations.—Heart-rate; blood-pressure tracing; urine flow (this may be counted or registered with an automatic drop recorder); inspection of kidney; color of kidney substance and of renal vein.

Apparatus.—Drum; manometer; ureter cannula; induction coil; injection buret.

Animal.—Morphinized dog or M. A. U. cat.

Operation.—Weigh, etherize; cannulae in carotid, tracheal and femoral vein. Expose kidney for observations. Insert ureter cannula.

Injections.—All into vein.

Experiment 1. Weak Stimulation of Vagus.

Experiment 2. Maximal Stimulation of Vagus.

Experiment 3. Cevadin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.: marked slowing or cardiac arrest, with prompt escape (veratrum viride acts similarly).

Experiment 4. Atropin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.: heart quickens; moderate rise of pressure. Try efficiency of vagus stimulation negative.

Experiment 5. Barium Chlorid.—Inject 20 mg. (2 c.c. of 1 per cent.) per kg., and repeat every ten minutes until death: the effects on the heart resemble those of digitalis, but the vasoconstriction is much more prominent and the pressure rises very high. The urine, however, is decreased, the renal arteries being also constricted. The intestines show violent peristalsis. This and the vasoconstriction are due to direct stimulation of the unstriated muscle.

QUESTIONS

(a) Describe the effects of cardiac slowing on the blood-pressure and kidney (Experiments 1 and 2).

(b) Ditto for cardiac quickening (Experiment 4).

(c) Describe the effects of cevadin, atropin, and barium.

(d) In how far are their effects explained by changes of heart-rate?

EXERCISE V.—(GROUP V) HEART-RATE AND RESPIRATION AS INFLUENCED BY BLOOD-PRESSURE

Changes in heart-rate are sometimes merely indirect results of changes of blood-pressure. Fast tracings should be taken at the critical points.

Distribution of Work.—Student A—Director and Reporter; calculates doses; takes notes and prepares report.

Student D—Chief Operator.

Student E—Assistant Operator; weighs animal; gives injections.

Student F—Anesthetist; artificial respiration; cleaning.

Student B—Pulse; blood-pressure tracings.

Student C—Respiratory tracings.

Observations.—Heart-rate; blood-pressure tracing; respiratory tracing.

Apparatus.—Manometer; respiratory tambour; injection buret; drum.

Animal.—Morphinized dog or M. A. U. cat.

Operation.—Weigh. Etherize. Cannulae in carotid, trachea, and femoral artery and vein. Small incision in abdomen to permit insertion of finger to compress aorta near diaphragm.

Injections.—All into vein.

Experiment 1. Nitroglycerin.—Inject 0.5 mg. ($\frac{1}{20}$ c.c. of 1 per cent.) per kg. Pay particular attention to heart-rate. (See Chapter XLIII, Exercise I, Experiment 4.)

Experiment 2. Hemorrhage.—Bleed animal so as to imitate the nitrite fall of pressure.

Experiment 3. Nitroglycerin and Compression of Aorta.—Inject as in Experiment 1, but keep blood-pressure level by appropriate compression of the aorta.

Experiment 4. Epinephrin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg. Pay particular attention to the heart-rate. (See Chapter XLIII, Exercise I, Experiment 9.)

Experiment 5. Compression of Aorta.—Compress aorta so as to imitate the epinephrin rise.

Experiment 6. Strophanthus and Hemorrhage.—Inject 1 mg. ($\frac{1}{10}$ c.c. of 1 per cent.) per kg. Bleed when necessary to keep blood-pressure level (compare with Exercise III, Experiment 5). Repeat every ten minutes till death.

QUESTIONS

(a) In how far may the nitrite tachycardia be explained by fall of blood-pressure. (Compare Experiments 1, 2, and 3.)

(b) Can the epinephrin slowing be explained by rise of blood-pressure? (Compare Experiments 4 and 5.)

(c) Can the strophanthus slowing be explained in this way? (Experiment 6.)

CHAPTER XLV

MYOCARDIAL DEPRESSANTS AND TONICS

EXERCISE I.—(GROUP I) CARDIAC DEPRESSANTS ON BLOOD-PRESSURE AND ORGAN VOLUME

(REPORTERS: F MEMBERS OF EACH GROUP)

Distribution of Work.—Student C—Chief Operator.

Student D—Assistant Operator; weighs animal; gives injections.

Student E—Anesthetist; artificial respiration; cleaning.

Student F—Director and Reporter; calculates doses; takes notes and prepares report.

Student A—Pulse; blood-pressure tracing.

Student B—Oncometer; inspection of intestinal vessels.

Observations.—Heart-rate; blood-pressure tracing; kidney or spleen oncometer; intestinal vessels. Draw out loop of intestine for inspection. Start tracing.

Animal.—Morphinized dog or M. A. U. cat.

Apparatus.—Damped mercury manometer; oncometer; injection buret.

Operation.—Weigh; etherize; tie on board. Cannulæ in carotid, trachea, and femoral vein. Expose kidney or spleen and place in oncometer.

Injections.—All into vein.

Experiment 1. Aconite (Therapeutic Dose).—Inject 5 mg. ($\frac{1}{20}$ c.c. of 10 per cent.) per kg.: slight slowing of the heart (stimulation of vagus centers) or no effect. Respiration increased (stimulation of center).

Experiment 2. Antipyrin.—Inject 100 mg. (1 c.c. of 10 per cent.) per kg. This illustrates the direct collapse action of "coal-tar" antipyretics.

Experiment 3. Phenol.—Inject 50 mg. (5 c.c. of 1 per cent.) per kg. (See Chapter XLIII, Exercise IV, Experiment 7.)

Experiment 4. Veratrum.—Inject 5 mg. ($\frac{1}{20}$ c.c. of 10 per cent.) per kg. (See Chapter XLIV, Exercise I, Experiment 3.)

Experiment 5. Aconite (Toxic Dose).—Inject 100 mg. (1 c.c. of 10 per cent.) per kg.: the heart is first slowed and strengthened (stimulation of vagus and myocardium); then weak and rapid (paralysis of vagus); then very irregular (overstimulation of myocardium); goes into delirium cordis and stops. The action may require half an hour.

Experiment 6. Chloroform Rigor.—Inject some chloroform into the peripheral end of one femoral artery: this causes immediate rigor of this leg.

QUESTIONS

- Describe effects of the drugs on blood-pressure and organ volume, and intestinal vessels.
- How far are the effects cardiac? Explain.
- In what pathologic conditions would cardiac depressants be useful?
- How could one treat cardiac collapse?

TECHNICAL REFERENCES

Heart Weight.—Joseph, 1908, Jour. Exp. Med., 10, 521; **Cardiac Stimulation**, Tigerstedt, 2, 4, 335; **Reflexes**, *ibid.*, 374; **Sounds**, *ibid.*, 195.

EXERCISE II.—(GROUP II). CIRCULATORY DRUGS ON ARTERIAL AND VEIN PRESSURE

The vein pressure is mainly an index of the efficiency of the circulation. It tends to rise when the heart is depressed; it tends to fall when the circulation is improved (Capps and Matthews).

Distribution of Work.—Student F—Director and Reporter; calculates doses; takes notes and prepares report.

Student C—Chief Operator.

Student D—Assistant Operator; weighs animal; gives injections.

Student E—Anesthetist; artificial respiration; cleaning.

Student A—Pulse; blood-pressure tracing.

Student B—Vein pressure.

Observations.—Heart-rate; blood-pressure tracing; vein pressure readings (after each experiment let some water run into the vein-manometer to flush blood out of the cannula). Transfer the readings to the proper places on the tracing.

Apparatus.—Blood-pressure; water-manometer for vein; induction coil; injection buret.

Animal.—Morphinized dog.

Operation.—Weigh, etherize, tie on board. Isolate vagus and place on thread. Cannulae in carotid, trachea, and both femoral veins (cardiac end). Connect one vein with water-manometer.

Injections.—Intravenous.

Experiment 1. Weak Vagus Stimulation.—Rise of vein pressure.

Experiment 2. Maximal Vagus Stimulation.—Rise of vein pressure.

Experiment 3. Nitroglycerin.—Inject 0.5 mg. ($\frac{1}{20}$ c.c. of 1 per cent.) per kg.: fall of vein pressure. (See Chapter XLIII, Exercise I, Experiment 4, p. 271.)

Experiment 4. Epinephrin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg.: vein pressure may rise.

Experiment 5. Ergot.—Inject 250 mg. (1 c.c. of 25 per cent.) per kg. (See Chapter XLIII, Exercise II, Experiment 5, p. 273.)

Experiment 6. Barium Chlorid.—Inject 20 mg. (2 c.c. of 1 per cent.) per kg. (See Chapter XLIV, Exercise IV, Experiment 5, p. 283.)

QUESTIONS

(a) Describe the effects of cardiac inhibition on vein pressure (Experiments 1 and 2).

(b) Describe the effects of the drugs on arterial and venous pressure.

(c) Which of the drugs would be useful, and which harmful, in dilation of the right heart or in venous hemorrhages?

EXERCISE III.—(GROUP III) CARDIAC STIMULANTS AND DEPRESSANTS ON CARDIOMYOGRAM

Distribution of Work.—Student F—Director and Reporter; calculates doses; takes notes and prepares report.

Student C—Chief Operator.

Student D—Assistant Operator; weighs animal; gives injections.

Student E—Anesthetist; artificial respiration; cleaning.

Student A—Pulse; blood-pressure tracing.

Student B—Cardiograph tracings.

Observations.—Heart-rate; blood-pressure tracing; myocardiograph tracing from ventricle and, if possible, from auricle; inspection of heart.

Apparatus.—Damped mercury manometer for blood-pressure tracing. Insufflation anesthesia (pp. 258, 259). Myocardiograph (Cushny, 1910, Heart, 2, 1). Induction coil.

Animal.—Morphinized dog or M. A. U. cat.

Operation.—As in Chapter XLII, Exercise V., adjusting the cardiograph instead of plethysmograph.

Injection.—All into femoral vein. Let conditions return to normal between the experiments.

Experiment 1. Caffein (Therapeutic Dose).—Inject 10 mg. (1 c.c. of 1 per cent.) per kg: increase of rate and excursions.

Experiment 2. Chloroform.—Let animal inhale Chloroform until there is a marked fall of blood-pressure: cardiac depression.

Experiment 3. Spartein.—Inject 5 mg. ($\frac{1}{2}$ c.c. of 1 per cent.) per kg. (See Chapter XLIV, Exercise II, Experiment 4, p. 281.)

Experiment 4. Digitalis.—Inject 50 mg. (1 c.c. of 5 per cent.) per kg. *Therapeutic stage of digitalis action:* Heart slowed, beats stronger (stimulation of cardiac muscle and vagus); blood-pressure high (cardiac effect and vasomotor stimulation); respiration increased (stimulation of center).

When this action has been observed (waiting twenty minutes if necessary), repeat the injection every fifteen minutes until death. *Toxic stage of digitalis*: the effects of toxic doses of digitalis on the circulation are extremely irregular, and may vary from moment to moment. The rate is generally increased, but may be slowed at times. The irregularities usually occur in groups; these are partly due to the influence of respiration (the reflex excitability of the vagus being heightened), partly to arrhythmia of the auricles and ventricles. The effects are based on an increased excitability of the cardiac muscle with systolic tendency, and on irregular activity of the vagus. Death occurs suddenly, sometimes by vagus stimulation, but more commonly by delirium cordis, the result of overstimulation of the heart. The blood-pressure may remain high until the end, or it may fall, according to the output of the heart and the persistence of the vasoconstriction.

Experiment 5. Caffein Rigor.—Inject 10 c.c. of 1 per cent. Caffein into the peripheral end of the femoral artery. Observe that this leg goes into rigor before the other (*drug rigor*).

QUESTIONS

Describe the effects of the drugs on the heart-rate, excursions, diastolic and systolic volume.

EXERCISE IV.—(GROUP IV) CIRCULATORY DRUGS ON PRESSURE IN PULMONARY ARTERY

The pressure in the pulmonary artery is determined mainly by the pressure in the right ventricle, and is, therefore, proportional to the vein pressure. It may also be influenced by the state of the pulmonary arterioles, but this is usually a minor factor.

The pressure in the pulmonary artery may therefore rise by cardiac insufficiency, by pulmonary vasoconstriction, or by extensive dilation of the systemic vessels. Decrease of pressure has the opposite explanations.

Distribution of Work.—Student F—Director and Reporter; calculates doses; takes notes and prepares report.

Student C—Chief Operator.

Student D—Assistant Operator; weighs animal; gives injections.

Student E—Anesthetist; artificial respiration; cleaning.

Student A—Pulse; blood-pressure tracing.

Student B—Tracings from pulmonary artery.

Observations.—Heart-rate; tracings of pressure in carotid and pulmonary artery.

Apparatus.—Two mercury manometers, writing above each other. Induction coil. Injection buret.

Animal.—Morphinized dog.

Operation.—Weigh, etherize, tie on board. Place vagus on thread. Cannulae in carotid, trachea, and femoral vein. Start artificial respiration. Open chest as described on pp. 258, 259. Place cannula into cardiac end of pulmonary artery of a lobe of the lung. Connect for tracings.

Injections.—Intravenous.

Experiment 1. Weak Vagus Stimulation.

Experiment 2. Maximal Vagus Stimulation.

Experiment 3. Nitroglycerin.—Inject 0.5 mg. ($\frac{1}{20}$ c.c. of 1 per cent.) per kg. (See Chapter XLIII, Exercise I, p. 271.)

Experiment 4. Epinephrin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg. (See Chapter XLIII, Exercise I, Experiment 9, p. 271.)

Experiment 5. Ergot.—Inject 250 mg. (1 c.c. of 25 per cent.) per kg. (See Chapter XLIV, Exercise IV, Experiment 5, p. 273.)

Experiment 6. Strophanthus.—Inject 1 mg. ($\frac{1}{10}$ c.c. of 1 per cent.) per kg. Repeat every ten minutes till death. (See Chapter XLV, Exercise III, Experiment 4, p. 286.)

QUESTIONS

(a) Describe and explain the effects of cardiac slowing on the pressure in the pulmonary artery (Experiments 1 and 2).

(b) Describe and explain the effects of the drugs on the carotid and pulmonary arterial pressures, and on the heart-rate.

(c) Which of these drugs would be useful, and which harmful, in dilation of the right heart?

(d) Ditto as to hemorrhage from rupture of a pulmonary artery?

EXERCISE V.—(GROUP V) CARDIAC DRUGS ON CARDIOPLETHYSMOGRAM

Distribution of Work.—Student F—Director and Reporter; calculates doses; takes notes and prepares report.

Student C—Chief Operator.

Student D—Assistant Operator; weighs animal; gives injections.

Student E—Anesthetist; artificial respiration; cleaning.

Student A—Pulse; blood-pressure tracing.

Student B—Tracing from cardioplethysmograph.

Observations.—Heart-rate; blood-pressure tracings; cardioplethysmogram; inspection of heart.

Apparatus and Operations.—As in Chapter XLII, Exercise VI, p. 263. Induction coil.

Animal.—Morphinized dog.

Injections.—Intravenous.

Experiment 1. Asphyxia and Recovery.—Arrest the flow of air. When the heart is materially weakened, restore the flow.

Experiment 2. Strychnin (Therapeutic Dose).—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg. (See Chapter XLIII, Exercise II, Experiment 1, p. 273.)

Experiment 3. Potassium Chlorid.—Inject 10 mg. (1 c.c. of 1 per cent.) per kg. Repeat every ten minutes if necessary. The heart will be somewhat weakened, slowed, and irregular (the pressure falling) and will stop rather suddenly (paralysis of cardiac muscle). (Magnesium produces similar effects.)

Experiment 4. Camphor.—Inject 5 mg. ($\frac{1}{2}$ c.c. of 1 per cent. in 40 per cent. alcohol) per kg.: usually little effect.

Experiment 5. Veratrum.—Inject 5 mg. ($\frac{1}{20}$ c.c. of 10 per cent.) per kg. (See Chapter XLIV, Exercise I, Experiment 3, p. 281.)

Experiment 6. Strophanthus.—Inject 1 mg. ($\frac{1}{10}$ c.c. of 1 per cent.) per kg. Repeat every ten minutes till death. (See Chapter XLV, Exercise III, Experiment 4, p. 286.)

QUESTIONS

(a) Describe the effects of the procedures and drugs on the blood-pressure, heart-rate, excursions, and systolic and diastolic volume.

(b) Which of the drugs might serve as cardiac stimulants, as cardiac depressants, and which are indifferent?

CHAPTER XLVI

INTESTINAL OSMOSIS-DIURESIS—TREATMENT OF ACUTE CARDIAC LESIONS

(REPORTERS: E MEMBERS OF EACH GROUP)

Introduction (Effects of Drugs on the Kidney).—The physiology and pharmacology of the kidneys differ conspicuously from that of the typical glands, such as the salivary: The kidney is not markedly affected by the usual glandular stimulants and depressants, such as pilocarpin and atropin. It functionates quite well when the nervous connections are divided. Its activity is most intimately connected with the state of the circulation. The *quantity of urine* is influenced mainly by the filtration pressure, *i. e.*, the difference between the pressure in the glomerular capillaries and in Bowman's capsule (cf. Chapter XXXV). This is determined by the systemic circulation, by the state of the vessels within the kidney, and by the viscosity of the blood. There is evidence that the kidneys possess an active vasodilator as well as a constrictor mechanism. The *composition of the urine* cannot be explained by a simple filtration theory. It necessitates the acceptance of unexplained forces. The changes occur by reabsorption and also by secretion.

The mechanism of urine secretion may be explained by several alternative theories, none of which is positively established to the definite exclusion of the others. The following working theory furnishes the most simple explanation of the phenomena: A physical filtration of urine occurs in the glomeruli. The filtrate probably corresponds to a protein-free plasma. The quantity of the filtrate depends mainly on the filtration pressure.

During the passage of the glomerular fluid through the urinary tubules a series of changes occur by the operation of powerful forces which cannot yet be explained on a physical basis. These cause the reabsorption of certain constituents and the secretion of others. The extent of these changes is indicated by the departure of the composition of the final urine from that of the protein-free blood plasma. It varies inversely to the rate of urine flow (a more rapid flow leaving less time for these changes). It is also influenced by the composition of the blood, but in a manner which is not fully understood.

The absorption involves mainly the water and chlorides; to a less extent the sulphates and phosphates; urea being the least absorbable constituent.

The secretion bears on the uric acid, certain pigments, and probably a variable proportion of the urea and of other urinary constituents.

Diuretics (drugs which increase the urine flow) may be grouped into the following classes:

Digitalis.—Acts by increasing the filtration pressure, through increased output of the heart, with stronger pulse-pressure; through lessened venous pressure; through the absorption of effusions, producing hydremic plethora. The diuretic tendency may be counteracted by constriction of the renal arterioles. It is, therefore, but little diuretic in health, but strongly so in cardiac disease, where the conditions for its favorable action are present.

Irritant Diuretics.—Volatile oils, calomel, alcohol, etc.; probably some of the salts, acids, and alkalis: small doses increase the vascularity and thereby the filtration pressure. It is possible that they also stimulate the secreting cells. Larger doses cause stasis and injury to the cells, and consequently lessened output of urine, with albuminuria, casts, and eventually anuria.

Irritant diuretics should not be used in nephritis.

Saline diuretics, including all substances which act by salt-action (water, non-toxic salt solutions, glucose, urea, etc.).—These produce *hydremic plethora*, *i. e.*, they dilute the blood. This increases the filtration pressure by increasing the total quantity of fluid; by lessening the viscosity and thereby reducing friction in the arterioles and capillaries; the lessened viscosity also reduces the filtration resistance. Stronger solutions further increase the filtration pressure by osmotic shrinkage of the renal cells. It is possible that some of these substances also stimulate the secreting cells or depress the reabsorption.

Stimulant Diuretics (Caffein, Theobromin, Theophyllin—Theocin).—These act directly on the kidney. They cause some dilation of the vessels, probably by shrinking the cells, and thereby increase the filtration pressure; but this is probably not the sole cause

of the diuresis. This is thought by some to involve a depression of the reabsorbing function; but it is more likely that they act by stimulating the secretory cells.

Drugs which constrict the vessels (suprarenal, barium, etc.) lessen the output of urine, the resistance in the afferent arterioles being increased more than the general blood-pressure. The effect of vasodilators is variable, according to whether they act more powerfully on the systemic or on the local vessels. In excised kidneys, vasoconstrictor drugs always lessen the urine flow, while vasodilators (cyanids) increase it.

TECHNICAL NOTES

Collection of Urine.—In operated animals cannulae are tied into the ureters (taking care that these are not kinked); or in rabbits, into the bladder (see Chapter XXXV). In survival experiments a permanent bladder fistula may be established (Schwarz and Wiechowski, 1914, *Zbl. Physiol.*, 28, 440).

Diuretic Factors.—In exact experiments the urine flow is referred to the weight of the animal. v. Schroeder selects the surplus excretion per 100 gm. of animal, calculated usually for one hour. Sollmann's factor relates to the maximal rate of secretion, being the maximum number of cubic centimeters of urine secreted in forty consecutive minutes per kilo of animal (*Amer. Jour. Physiol.*, 1903, 9, 454).

TREATMENT OF CARDIAC LESIONS

The acute lesions produced experimentally are not strictly analogous in their effects to the usual chronic clinical lesions. However, the principles illustrated in these experiments are fairly applicable to both.

TECHNICAL REFERENCES TO CARDIAC LESIONS

General Technic.—Rosenbach, *Arch. exp. Path.*, 9, 1; Emerson, 1907, *Experimental Pathologic Lesions*, N. Y. Med. Jour., April 20.

Temporary Valvular Lesions.—Wiggers and Du Bois, 1913, *Soc. Exp. Biol. Med.*, 10, 87.

Aortic Insufficiency.—Acute, Zollinger, 1909, *Arch. exp. Path. Pharm.*, 61, 193; Hasenfeld and Romberg, 1897, *Arch. exp. Path.*, 37, 333.

Mitral Stenosis.—Hirschfelder, 1908, *John Hopkins Hosp. Bul.*, 19, 319.

Myocarditis.—Fleisher and Loeb, *Arch. Int. Med.*, Feb., 1909; *ibid.*, 1910, 6, 427 (Epinephrin with Spartein or Caffein).

Experimental Surgery of Heart.—Werelius, 1914, *Jour. Amer. Med. Assoc.*, 63, 1338.

Electrocardiograms.—Tigerstedt, 24, 203; Interpretation, Eyster and Meek, 1913, *Arch. Int. Med.*, 11, 204; Pardee, 1914, *Jour. Amer. Med. Assoc.*, 62, 1311; Waller, 1914, *Harvey Lectures*, p. 17. Protection of *string galvanometer* against external electric disturbances, H. B. Williams, 1916, *Amer. Jour. Physiol.*, 40, 230.

Pressure in Cardiac Cavities.—Tigerstedt, 24, 205; Heinz, 1, 850.

Movements of Cardiac Valves.—Dean, 1915, *Soc. Exp. Biol. Med.*, 13, 5.

DISTRIBUTION OF WORK

Student E—Director and Reporter; calculates doses; takes notes and prepares report.

Student B—Chief Operator.

Student C—Assistant Operator; weighs animal; gives injections.

Student D—Anesthetist; artificial respiration; cleaning.

Student F—Pulse; blood-pressure tracing.

Student A—All other observations.

Animals.—Morphinized dogs.

Injections.—All intravenous.

EXERCISE I.—(GROUP I) FIRST PART: DIURETICS; URINE FLOW, BLOOD-PRESSURE, AND RESPIRATION; SECOND PART: AORTIC STENOSIS WITH CARDIOPLETHYSMOGRAM

FIRST PART: DIURESIS

Observations.—Heart-rate; blood-pressure tracing; urine flow (this may be counted or registered with an automatic drop recorder); inspections of kidney; color of kidney substance and of renal vein. Respiratory tracing.

Apparatus.—Manometer; ureter cannula; injection buret; respiratory tambour; drum induction coil.

Operations.—Weigh; etherize; cannulæ in carotid, trachea, and femoral vein. Expose kidney for observations. Insert ureter cannula. Connect tracheal tambour for respiratory tracing.

Experiment 1. Sulphate Diuresis.—Inject 25 c.c. per kg. of warm sodium sulphate (2.5 per cent. of dried or 5 per cent. of crystals). Collect the urine after a few minutes. Rise of blood-pressure, stronger and usually slower heart, increased oncometer and respiration. The effect is usually short, and may be small, especially if the animal is in good condition. (Stimulation of the medullary centers and cardiac muscle by the increased quantity of blood, and by salt action.) The urine flow is promptly increased, and remains high for a considerable time (dilution of blood, lessened viscosity, increased quantity of blood in vessels, "hydremic plethora"). Note that the carotid pressure is not increased sufficiently to account for the diuresis. The volume of the kidney increases.

Some animals do not show any diuresis, especially if the kidneys have been injured. Should this be the case, the ureter observations may be abandoned, and replaced by myocardiogram, oncometer, or respiratory tracings.

Test the urine for chlorids ($\text{HNO}_3 + \text{AgNO}_3$), comparing it with the original bladder-urine. The chlorid has almost disappeared (due to dilution of the plasma; the chlorid could be made to reappear by the injection of sodium nitrate, iodid, bromid, or sulfocyanid. These act probably by liberating the "combined" chlorid of the plasma).

The hypodermic or intravenous injection of normal saline solution or the drinking of water increase the diuresis in the same manner as the sulphate solution. The latter would not be diuretic by mouth, as it is but imperfectly absorbed.

Experiment 2. Epinephrin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg. (See Chapter XLIII, Exercise I, Experiment 9, p. 271.)

Experiment 3. Spartein.—Inject 5 mg. ($\frac{1}{2}$ c.c. of 1 per cent.) per kg. (See Chapter XLIV, Exercise II, Experiment 4, p. 281.)

SECOND PART: AORTIC STENOSIS

Observations, Apparatus, and Operation.—(See Chapter XLII, Exercise VI, p. 263.)

Experiment 4. Aortic Stenosis.—Place screw-clamp on aorta, as near as possible to its origin, and tighten while taking tracing, so that the pulsations of the manometer are materially reduced, but not abolished.

Experiment 5. Weak Vagus Stimulation.

Experiment 6. Strong Vagus Stimulation.

Experiment 7. Saline Infusion.—Inject slowly warm N. S., 25 c.c. per kg.

Experiment 8. Strophanthus.¹—Inject Strophanthus as in Chapter XLIV, Exercise I, Experiment 6, p. 281.

¹ Aortic Compression and Strophanthin, de Heer, 1912, Arch. ges. Physiol., 148, 1.

QUESTIONS

- (a) Describe and explain the effects of the procedures of Experiments 1 to 3 on the blood-pressure and ureter flow.
- (b) Which of these would be useful in dropsy?
- (c) Which in uremia?
- (d) Describe and explain the effects of aortic stenosis.
- (e) How is this modified by the procedures of Experiments 5 to 8?
- (f) Which of these would be useful, which harmful, and which indifferent?

EXERCISE II.—(GROUP II) FIRST PART: URINE FLOW. SECOND PART: HYDROPERICARDIUM

FIRST PART: URINE FLOW

Observations.—Heart-rate; blood-pressure tracing; urine flow (this may be counted or registered with an automatic drop-recorder); inspection of kidney; color of kidney substance and of renal vein.

Apparatus.—Drum; manometer; ureter cannula; drop-recorder; injection buret; induction coil; dish, rod, funnel and strainer for defibrinated blood.

Operation.—Weigh; etherize; cannulae in carotid, trachea, and femoral vein. Expose kidney for observation. Insert ureter cannula.

Experiment 1. Absorption of Sodium Chlorid and Magnesium Sulphate.—Make a 2-inch incision in linea alba, draw out a loop of intestine, and ligature it in two places, about 25 cm. apart. Make an opening just inside one of the ligatures. Strip the piece of intestine of its contents, insert the end of a funnel into the opening, and allow a measured quantity of $MgSO_4$ solution (3.6 per cent. of the dried salt at $110^\circ C.$) to flow in. Withdraw the funnel and tie off the opened portion. Replace the loop of intestine and draw forth another loop; treat this loop also, using 1 per cent. $NaCl$ instead of $MgSO_4$, and sew up the wound. The $NaCl$ and $MgSO_4$ solutions have the same freezing-point. Leave until all the other experiments are finished, then open the abdomen, find the ligated intestines, and measure their contents: the $MgSO_4$ has not diminished as much as the $NaCl$, because the former salt is not readily absorbed and retains water by salt action.

Experiment 2. Saline Diuresis.—Inject warm 1 per cent. $NaCl$, 25 c.c. per kg. (See Exercise I, Experiment 1.)

Experiment 3. Strong Vagus Stimulation.

Experiment 4. Theobromin-sodium Salicylate.—Inject 20 mg. ($\frac{1}{3}$ c.c. of 10 per cent.) per kg. The urine flow increases. Note that the changes in the carotid pressure do not suffice to explain the diuresis. The effects on the circulation are identical with those of carotid. The volume of the kidney increases.

Experiment 5. Hemorrhage.—Withdraw about 25 c.c. per kg. of blood from the femoral artery while taking a tracing. (The blood is to be whipped vigorously with a glass rod for about ten minutes, or until thoroughly defibrinated, strained through muslin, and heated to $40^\circ C.$)

The ureter flow stops as the pressure falls. The heart-beats are quickened and weakened. The respiration is dyspneic.

The cardiac and respiratory effects are due to anemic depression of the vagus and respiratory centers. The anuria is explained by the low blood-pressure.

Observe the pressure for some five minutes after the completion of the hemorrhage: there is a slight, but very imperfect recovery.

Experiment 6. Injection of Normal Saline Solution.—*Urine Flow, Blood-pressure.*—Inject 25 c.c. per kg. of warm normal saline solution. The urine flow and the blood-pressure recover considerably, but do not usually reach the original level. The effect lasts for several hours. Note the much larger effect as compared with saline injection in the normal animal.

Experiment 7. Injection of Defibrinated Blood.—*Urine Flow, Blood-pressure.*—After fifteen minutes inject the warmed defibrinated blood: the ureter flow and blood-pressure recover completely.

SECOND PART: HYDROPERICARDIUM

Observations.—Pulse-rate; blood-pressure tracing; inspection of heart.

Operation.—Start artificial respiration. Expose the heart as in Chapter XLII, Exercise V. Tie a cannula into the apex of the pericardium. Connect with reservoir of saline.

Experiment 8. Pericardial Pressure.—Study effects of increasing the pressure by raising the reservoir. Leave this at a level which produces fairly serious interference with the heart.

Experiment 9. Weak Vagus Stimulation.

Experiment 10. Strong Vagus Stimulation.

Experiment 11. Saline Infusion.

Experiment 12. Strophanthus. (See Exercise I, Experiments 5 to 8.)

Experiment 13. When the animal is dead, complete Experiment 1.

QUESTIONS

- (a) Describe and explain the effects of the procedures of Experiments 2 to 7 on the blood-pressure and ureter flow.
- (b) Which of these would be useful in dropsy?
- (c) Which in uremia?
- (d) Describe and explain the effects of pericardial effusion.
- (e) How is this modified by the procedures of Experiments 9 to 12?
- (f) Which of these would be useful, which harmful, and which indifferent?
- (g) Explain how Epsom salt increases the bulk of the feces.

EXERCISE III.—(GROUP III) FIRST PART: URINE FLOW AND KIDNEY VOLUME. SECOND PART: MYOCARDITIS

FIRST PART: URINE FLOW AND KIDNEY VOLUME

Observations.—Heart-rate; blood-pressure tracing; ureter flow; kidney volume; inspection of intestinal vessels.

Apparatus.—Drum; manometer; ureter cannula; oncometer; injection buret; induction coil.

Operation.—Weigh; etherize; tie on board. Thread under vagus. Cannulae in carotid, trachea, and femoral vein. Expose kidney and place in oncometer. Tie ureter cannula in other ureter. Place loop of intestine for inspection.

Experiment 1. Absorption of Sodium Chlorid and Magnesium Sulphate.—(See Exercise II.)

Experiment 2. Hypertonic Salt Diuresis.—Inject slowly 10 per cent. NaCl, 2.5 c.c. per kg. Compare results with Experiment 2 of Exercise II. The same quantity of NaCl is used, but the concentration is different.

Experiment 3. Strong Vagus Stimulation.

Experiment 4. Theophyllin-sodium Acetate.—Inject 10 mg. (1 c.c. of 1 per cent.) per kg. Results similar to theobromin (see Exercise II, Experiment 4).

SECOND PART: ACUTE MYOCARDITIS

Operation.—Start artificial respiration and expose heart as in Chapter XLII, Exercise V.

Observations.—Heart-rate; blood-pressure tracing; inspection of heart.

Experiment 5. Injection of Alcohol.—Inject 2 c.c. of 95 per cent. Alcohol into myocardium. Repeat several times until the blood-pressure has fallen markedly.

Experiment 6. Weak Vagus Stimulation.

Experiment 7. Strong Vagus Stimulation.

Experiment 8. Saline Infusion.

Experiment 9. Strophanthus.—(See Exercise I, Experiments 5 to 8.)

Experiment 10. When the animal is dead, complete Experiment 1.

QUESTIONS

(a) Describe and explain the effects of the procedures of Experiments 2 to 4 on the blood-pressure and ureter flow.

(b) Which of these would be useful in dropsy?

(c) Which in uremia?

(d) Describe and explain the effects of acute myocardial degeneration.

(e) How is this modified by the procedures of Experiments 6 to 9?

(f) Which of these would be useful, which harmful, and which indifferent?

(g) Explain how Epsom salt increases the bulk of the feces.

EXERCISE IV.—(GROUP IV) FIRST PART: URINE FLOW AND KIDNEY VOLUME. SECOND PART: AORTIC ANEURYSM

FIRST PART: DIURESIS

Observations.—Heart-rate; blood-pressure tracing; ureter flow; kidney volume; inspection of intestinal vessels.

Apparatus.—Drum; manometer; ureter cannula; oncometer; injection buret; induction coil.

Operation.—Weigh; etherize; tie on board. Thread under vagus. Cannulae in carotid, trachea, and femoral vein. Expose kidney and place in oncometer. Tie ureter cannula in other ureter. Place loop of intestine for inspection.

Experiment 1. Glucose Diuresis.—Inject warm 6 per cent. solution, 25 c.c. per kg. (See Exercise I, Experiment 1.)

Experiment 2.—Amyl Nitrite.—Administer by inhalation. (See Chapter XLIII, Exercise I, Experiment 4.)

Experiment 3. Caffein.—Inject 10 mg. (1 c.c. of 1 per cent.) per kg. (See Exercise II, Experiment 4.)

Experiment 4. Hemorrhage. Experiment 5. Saline. Experiment 6. Re-injection of Blood.—See Exercise II, Experiments 5, 6, and 7.

SECOND PART: AORTIC ANEURYSM

Experiment 7. Aortic Aneurysm.—Tie into cardiac end of other carotid a cannula, the free end of which communicates with a fairly strong but extensible rubber bulb (made from the finger of a rubber glove, well oiled).

Remove clamp from artery. This simulates a pulsating aneurysm. It should be watched during the experiment.

Experiment 8. Weak Vagus Stimulation. Experiment 9. Strong Vagus Stimulation. Experiment 10. Saline Injection. Experiment 11. Strophanthus.—(See Exercise I, Experiments 5 to 8.)

QUESTIONS

(a) Describe and explain the effects of the procedures of Experiments 1 to 6 on the blood-pressure and ureter flow.

(b) Which of these would be useful in dropsy?

(c) Which in uremia?

(d) Describe and explain the effects of aortic aneurysm.

(e) How is this modified by procedures of Experiments 8 to 11?

(f) Which of these would be useful, which harmful, and which indifferent?

EXERCISE V.—(GROUP V) FIRST PART: URINE FLOW AND KIDNEY VOLUME. SECOND PART: CORONARY SCLEROSIS (CARDIOMYOGRAM)

FIRST PART: DIURESIS

Observations.—Heart-rate; blood-pressure tracing; ureter flow; kidney volume; inspection of intestinal vessels.

Apparatus.—Drum; manometer; ureter cannula; oncometer; injection buret; induction coil.

Operations.—Weigh; etherize; tie on board. Thread under vagus. Cannulae in carotid, trachea, and femoral vein. Expose kidney and place in oncometer. Tie ureter cannula in other ureter. Place loop of intestine for inspection.

Experiment 1. Absorption of Sodium Chlorid and Magnesium Sulphate.—(See Exercise II, Experiment 1.)

Experiment 2. Saline Diuresis.—Inject, 25 c.c. per kg. of warm Locke solution (without glucose). (See Exercise I, Experiment 1.)

Experiment 3. Epinephrin.—Inject 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) per kg. (See Chapter XLIII, Exercise I, Experiment 9, p. 271.)

Experiment 4. Pituitary.—Inject solution, 0.1 c.c. per kg. (See Chapter XLIII, Exercise II, Experiment 4, p. 273.)

SECOND PART: CORONARY SCLEROSIS

Observation, Apparatus, and Operation for Myocardiogram.—(See Chapter XLIV, Exercise I, p. 281.)

Experiment 5. Coronary Sclerosis.—With a hypodermic syringe inject a suspension of lycopodium¹ into a coronary artery.

(Optional) For this may be substituted:

Auricular Fibrillation.—Electric stimulation of auricle.

Delirium Cordis.—Electric stimulation of the middle third of the anterior coronary artery (Kronecker).

Experiments 6 and 7. Weak and Strong Vagus Stimulation.

Experiment 8. Inhalation of Amyl Nitrite.

Experiment 9. Strophanthus.—Inject Strophanthus, as in Chapter XLIV, Exercise I, Experiment 6, p. 281.

Experiment 10. When the animal is dead, complete Experiment 1.

¹ *Lycopodium Suspension.*—Lycopodium spores heated and shaken with normal saline, to form a thick cream.

QUESTIONS

- (a) Describe and explain the effects of the procedures of Experiments 2 to 4 on the blood-pressure and ureter flow.
- (b) Which of these would be useful in dropsy?
- (c) Which in uremia?
- (d) Describe and explain the effects of coronary obstruction.
- (e) How is this modified by the procedures of Experiments 6 to 9?
- (f) Which of these would be useful, which harmful, and which indifferent?
- (g) Explain how Epsom salt increases the bulk of the feces.

EXERCISE VI.—(OPTIONAL) FATE OF INJECTED SALT SOLUTION

Anesthetize a dog. Place cannulae into trachea, carotid artery, femoral vein, both ureters, and ileum.

Draw a sample (5 or 10 c.c.) of blood, defibrinate, and set aside for the determination of the ratio of corpuscles and plasma (see Index).

Inject into the vein a 0.9 per cent. NaCl solution, 25 c.c. per kg., in ten minutes. At the end of the injection draw another sample of blood (defibrinate), and again in half an hour and in two hours. Collect the urine and the intestinal fluid during the same periods. Kill the animal and measure the fluid in the intestines, pleura, and peritoneum. Note whether the liver and lungs are edematous.

Determine the ratio of corpuscles in each of the blood samples. Assuming that the original volume of blood was 75 c.c. per kg., calculate from these data the distribution of the injected fluid at each of the periods (Sollmann, 1901, Arch. exp. Path., 46, 1).

Sodium sulphate or hypertonic solutions may be used; or *gelatin solution*, which leaves the vessels more slowly (1.5 gm. gelatin, melted with 100 c.c. of water, and mixed with 1000 c.c. of 0.9 per cent. NaCl and 2 gm. of sodium carbonate, Hogan, 1915, Jour. Amer. Med. Assoc., 64, 721).

The *Lymph* may also be studied (Heinz, 2, 335).

EXERCISE VII.—(OPTIONAL) ANASARCA

The injection of excessive quantities of saline solution into normal animals produces ascites, but not anasarca (Cohnheim and Lichtheim). True anasarca occurs if saline solution is injected into an animal poisoned by arsenic (Magnus); or locally, if the skin is irritated by iodine or hot water.

APPENDIX

APPENDIX A.—ARRANGEMENT AND GENERAL EQUIPMENT OF LABORATORIES

THE LABORATORY ROOMS

THE pharmacology courses may be given in the chemic, pharmaceutic, and physiologic laboratories if no other arrangement can be made; but the efficiency of the teaching and research is undoubtedly enhanced by separate rooms and equipment. The laboratory should consist of a chemic and animal department, preferably in adjacent rooms. The materia medica collection may be placed in the chemical room or in a convenient corridor. Additional rooms for lectures, research, toxicology, storage, for the keeping and observation of animals, etc., are highly desirable. They should be in close vicinity; the animal rooms, however, will be less annoying in another part of the building.

EQUIPMENT OF THE CHEMICAL DEPARTMENT

This should contain the chemic tables, lockers, and sinks for the students; a fume-chamber; balance and druggists' scales; and a moderate equipment of chemic apparatus.

The **chemic tables** may be of any of the varieties used in chemic laboratories. A height of 3 feet is convenient. A working space of 6 by 2 feet and a single locker suffice for each pair of students. The lockers should be of the height of the table, $2\frac{1}{2}$ feet wide, with a shelf 9 inches from the top. It is cheap and convenient to have $\frac{1}{4}$ -inch iron rods fixed to the tops of the tables for clamping retort rings, etc.

EQUIPMENT OF THE ANIMAL DEPARTMENT

This should be equipped with a large demonstration table and case of demonstration apparatus; sinks; easily movable tables and lockers for students' work; shelves for reagents; a chemic bench; drawers for supplies, etc.

Tables for Animal Work.—These may be of pine, strongly built, 3 feet high by 6 feet long and 2 feet wide; $1\frac{1}{2}$ inch top; solid legs. Drawers are rather objectionable. Two tables are needed for six students. In operative experiments the two tables are set in the form of a **T**, the lower table being used for operating, the upper one for apparatus.

Black Stain for Table Tops.—The clean table is given two coats of the following solutions: No. 1, applied hot, the second as soon as the first is dry. This is followed by two coats of Solution No. 2; this is allowed to dry thoroughly (one to two days) and sand-papered lightly. It is then paraffined with floor-wax.

Solution No. 1:

Copper sulphate.....	1 part
Potassium chlorate.....	1 part
Water.....	8 parts

Boil for five minutes.

Solution No. 2:

Anilin hydrochlorate.....	3 parts
Water.....	20 parts
Or,	
Anilin (liquid).....	6 parts
Hydrochloric acid.....	9 parts
Water.....	50 parts

The **lockers** (one for six students) may be placed at the side of the room near the tables. There should also be an open shelf for special apparatus.

Apparatus.—It is advisable to buy as much as possible of manufactured apparatus of the best quality which the resources will allow. The satisfaction of working with instruments which give accurate and trustworthy results, the training in exactness, and the practice with apparatus such as would actually be used in research are advantages which offset, in most cases, those of home-made apparatus. The latter, however, have some valuable qualifications besides cheapness, especially in that they encourage independence and ingenuity. A certain amount of home-made apparatus is therefore very useful, especially if time permits the students to manufacture it themselves.

APPENDIX B.—EQUIPMENT OF CHEMIC LOCKERS (FOR EACH PAIR OF STUDENTS)

2 Bunsen burners and tubing.	1 Mortar and pestle, 10 cm.
1 Retort stand.	2 Keys.
2 Retort rings.	1 Requisition pad.
1 Tripod.	1 Percolating tube.
1-liter wash bottle.	1 Water-bath with rings.
2 Evaporating dishes (10 cm.).	1 100-c.c. graduate.
1 Evaporating dish (400 c.c.).	1 Pint percolator.
2 Funnels, 6 cm.	1 Pill tile.
1 Funnel, 12 cm.	1 Pill box.
5 Beakers, 25-150 c.c.	1 Powder box.
4 Flasks, 250 c.c.	1 Steel spatula.
2 Tumblers.	1 Horn spatula.
50 Test-tubes.	1 Thermometer, 0-100°.
2 Test-tube racks.	1 25-c.c. Conic graduate.
2 Test-tube brushes.	3 Watch-glasses, 1½ inches.
2 Test-tube clamps.	1 Sponge.
2 Slide clamps.	1 Towel.
1 Earthen jar.	

The following are not charged:

Filter-paper; label paper; wire gauze; glass slides, tubing, rods, pipets, etc.

APPENDIX C.—REAGENTS NEEDED FOR CHEMIC EXERCISES

The reagents employed in pharmacology are so numerous that the problem of keeping them conveniently accessible is quite serious. It will be found convenient to divide them into three classes: (A) for every three students; (B) for every six students; and (C) for every six students for special experiments. (A) and (B) should be arranged in alphabetic order on the shelves of the chemic tables. (C) may be arranged by the exercise numbers, and kept on a side shelf when not in use.

It will be found very advantageous to number the containers and their places, and to demand that every reagent be replaced in proper order as soon as used.

A number of the solutions are perishable and should not be kept over a year. These are marked * in the following lists. Others (**) should be furnished fresh for each exercise. It is well to distinguish these by colored labels (green for * and red for **) for the ready guidance of the laboratory assistant. He can save himself some labor by keeping concentrated stock solutions on a special shelf.

LIST A.—COMMON CHEMIC REAGENTS

Kept on shelves of chemic tables (50 to 100 c.c. of each). For three students:

Acid, Acetic, 5 per cent.	Iodin in KI, 1 per cent. of iodine, KI q. s. to dissolve
Acid, Hydrochloric, Conc., C. P.	Lead Acetate, 5 per cent.
Acid, Hydrochloric, 5 per cent.	Litmus Paper.
Acid, Nitric, Conc., C. P.	Magnesia Mixture. ¹
Acid, Picric, Saturated Aqueous.	Magnesium Sulphate, powdered.
Acid, Sulphuric, Conc., C. P.	Mercuric Chlorid, 1 per cent.
Acid, Sulphuric, 5 per cent.	Mercuric-potassic Iodid (Mayer's Reagent). ²
Alcohol, Ethyl, 95 per cent.	Oleum Olivæ or Gossypii (cotton- seed).
Ammonia Water, 10 per cent.	Potassic Bichromate, Saturated (about 3½ per cent.).
Ammonium Sulphate, Powdered.	Potassic Ferricyanid, 5 per cent.
Barium Chlorid, 5 per cent.	Potassic Ferrocyanid, 5 per cent.
Barium Hydrate, Saturated Aqueous.	Potassic Iodid, 3 per cent.
**Bromine Water, Saturated Aqueous.	Silver Nitrate, 1 per cent.
Calcium Chlorid, 1 per cent.	Sodium Acetate, 5 per cent.
Calcium Hydrate (Lime Water), Saturated Aqueous.	Sodium Carbonate, 5 per cent.
Chloroform.	Sodium Chlorid, crystals
Cupric Sulphate, 5 per cent.	Sodium Hydrate, 10 per cent.
Ether.	Sodium Phosphate, 5 per cent.
Ferric Chlorid, 0.1 per cent.	Sodium Sulphate, powdered.
*Ferrous Sulphate, 1 per cent.	
Glycerin.	

* (Green Label) should not be kept over a year.

** (Red Label) should be freshly made.

¹ *Magnesia Mixture:*

MgSO ₄ , Crystals	1
NH ₄ Cl	1
NH ₃ (10 per cent.)	4
Water	8

² *Mercuric-potassic Iodid (Mayer's Reagent):*

HgCl ₂	13.55 gm.
KI	49.80 Gm.
Water	q. s. 1.0 liter.

LIST B.—ON TOP SHELF OF CHEMIC TABLES. FOR SIX STUDENTS

("Pd." stands for "powdered"). Approximate amounts (grams or cubic centimeters):

Acacia, granulated.....	20
Acetanilid.....	20
Acid, Boric, Pd.....	20
Acid, Phosphotungstic (10 per cent. in 4 per cent. HCl).....	10
Acid, Sulphuric-ferric (1 : 1000, Ferric Chlorid).....	10
Acid, Tartaric, Pd.....	10
Alum, Pd.....	10
Antipyrin.....	10
Bismuth Subcarb.....	20
Caffein.....	1
Calomel.....	10
Chloral.....	5
Creta prepar. (Chalk).....	25
Ferric Ammon. Citrate, 5 per cent.....	25
Ferric Chlorid, Tr.....	25
Formaldehyd, Liq.....	20
Fuller's Earth.....	25
Gasolin.....	50
Glucose, Pd.....	20
*Guaiac, Tr.....	10
Hexamethylenamin.....	10
*Hydrogen Peroxid.....	20
Iodin, Tr.....	10
Lead Subacetate Sol.....	25
Methyl Alcohol.....	20
Millon's Reagent ¹	20
Morphin Sulph.....	0.1
Phenol Liq.....	25
*Phenol, 5 per cent.....	25
Potas. Arsenite, Liq.....	20
Potas. Bichromate, Pd.....	5
Potas. Bromid, Pd.....	20
Potas. Chlorate, Pd.....	10
Potas. Nitrate, Pd.....	10
Potas. Oxalate, Pd.....	10
Potas. Permanganate, 1 per cent.....	25
Quinin Sulphate, Pd.....	0.1
*Quinin Sulphate, 0.1 per cent. acidulated, aqueous.....	25
*Quinin Sulphate, saturated aqueous.....	25
Resorcin, Pd.....	0.5
Sand.....	100
Sod. Acetate, Pd.....	10
Sod. Benzoate, Pd.....	10
Sod. Bicarbonate.....	20
Sod. Borate, Pd.....	20
Sod. Nitrite, Pd.....	10
Sod. Salicylate, Pd.....	10

¹ *Millon's Reagent*: Dissolve 1 part of metallic mercury in 1 part by weight of cold fuming nitric acid, cool, and dilute with 2 parts of distilled water. Decant from the sediment. The solution contains mercuric and mercurous nitrate.

Sod. Thiosulphate (Hyposulphite), Pd.	10
Spir. Nitrous Ether.	20
Starch.	100
Strychnin Sulphate, Pd.	0.1
Sugar, Cane, Gran.	200
Talc, Purif.	50
Tannin, Pd.	10
Turmeric Paper	
Turpentine Oil.	50

LIST C.—SPECIAL REAGENTS, ARRANGED BY EXERCISES

This does not include optional experiments, demonstrations, or special assignments.

The quantities are for six students.

CHAPTER I

*Nicotin, 1 per cent.	5
*Salicin, 1 per cent.	10
Licorice, Fldext.	20
Licorice, Fldext, Acidulated.	20
Soap-bark, Tr.	10
**Rhubarb Infus., 5 per cent.	25
**Cinchona Infus., 5 per cent.	25
**Acacia, 10 per cent.	25
Rosin, Pd.	20

CHAPTER II

Cinnamon Oil, in drop bottles.	5
*Quick-lime, in 3-gm. portions.	3
Quart bottles.	3
Alcohol.	250
Peppermint Oil.	5
Peppermint Herb, in 0.1-gm. portions.	3
Digitalis, in 1.5-gm. portions.	3
*Cinnamon Water.	100
Arnica, in 10-gm. portions.	3
Cinchona, Pd., in 20-gm. portions.	3
Cod-liver Oil.	30
Syrup.	25
Glycyrrhiza, Pd., in 2-gm. portions.	6
Excipient, or Glycerite Acacia.	5
Powder Papers.	60
Capsules, No. 3.	60
Zinc Oxid, Pd., in 2-gm. portions.	6
Benzoinated Lard, in 10-gm. portions.	6
Flaxseed, Ground.	300

CHAPTER III

*Strychnin Sulphate, 1 per cent.	10
Spir. Ammon. Arom.	25
Myrrh, Tr.	25
*Acaciæ, Mucil.	25
Sod. Chlorid, Sat. Sol.	25

CHAPTER V

**Strychnin Sulphate, 1 : 50,000.....	30
**Sod. Iodate, 1 per cent.....	10
**Starch Paste, 2 per cent.....	25
**Marquis Reagent.....	10
Ammonium Molybdate.....	1
Morphin Tablets, $\frac{1}{8}$ grain.....	12
Opium, Tr.....	10
Apomorphin Hydrochlorid.....	0.1
Apomorphin Hydrochlorid, 1 : 500.....	10
Atropin.....	0.1
**Epinephrin, 1 : 50,000.....	10
**Aconite, 1 : 300.....	30
Veratrin.....	0.1

CHAPTER VII

**Calx Chlorinata, 5 per cent.....	10
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CHAPTER VIII

**Formaldehyd, 1 : 50,000.....	25
*Jorissen Phloroglucin Reagent.....	15
*Phenylhydrazin HCl, 0.5 per cent.....	10
*Sod. Nitroprussid, 5 per cent.....	10
**Milk.....	50
**Milk with Formaldehyd, 0.1 c.c. of the Solution per liter.....	50
*HCN, 1 : 1000.....	5

CHAPTER XIII

*Sugar, 1 per cent.....	50
Sod. Saccharin, 0.1 per cent.....	50
Glycerin, 10 per cent.....	50
*Lactose, 10 per cent.....	50
*Glucose, 10 per cent.....	50
*Levulose, 10 per cent.....	50
Magnes. Sulph., 20 per cent.....	100
KBr, 5 per cent.....	100
Sod. Salicylate, 10 per cent.....	100
Chloral, 10 per cent.....	100
Quinin Bisulph., 1 per cent.....	100

Each of the following 25 c.c.:

Magnes. Sulph., 2% in water; in milk; in 5% acacia; in syrup.

KBr, 0.5% in water; " " " "

Sod. Salicyl., 1% in water; " " " "

Ammon. Chlorid, 0.5% in water; " " " "

Chloral, 1% in water; " " " "

Quinin Bisulph., 0.1% in water; " " " "

Also above list in Syr. Citric Acid; Syr. Glycyrrhizæ; Elixir;

Comp.Tr. Cardamom; Syr. Eriodictyon.

Saccharin, 0.1 per cent.....	25
Saccharin, 0.01 per cent.....	25
Cod-liver Oil.....	25
Cod-liver Oil with 0.4 per cent. Oil Peppermint.....	25
Cod-liver Oil with 0.4 per cent. Oil Lemon.....	25

Cod-liver Oil, 50 per cent. Emulsion, not flavored.....	25
Chalk.....	10
Chalk, 5; Milk-sugar, 5	
Chalk, 5; Cane-sugar, 5	
Chalk, 5; Cane-sugar, 3; Cacao, 2	
Chalk, 5; Cane-sugar, 4; Cinnamon, 1.	
Euquinin (Quinin-ethyl Carbonate).....	1
Quinin-Fuller's Earth Precipitate.....	1
Quinin Tannate.....	1
Quinin Alkaloid.....	1
Quinin Sulphate.....	1
Magnes. Sulph., 5 per cent.....	25
Sod. Sulph., 5 per cent.....	25
Sod. Phosphate, 5 per cent.....	25
Sod. Pot. Tartrate, 5 per cent.....	25

CHAPTER XV

Weighed drugs for each exercise.

CHAPTER XVI

*Strychnin Sulph., 0.1 per cent.....	10
*Morphin Sulph., 0.1 per cent.....	10
**Infusion Tea, 5 per cent.....	25
**Infusion Coffee, 5 per cent.....	25
**Egg-white Solution, 1 : 5.....	25
Phosphorus, 10 small pieces, shot size.	

CHAPTER XVII

25 c.c. of each of the following, in wide-mouth jars:

*Citric acid, 1 per cent. in water.	} *Ditto in 10 per cent. starch paste.	
*Quassia, $\frac{1}{10}$ per cent. in water.		
Quinin Bisulph., $\frac{1}{10}$ per cent. in water.		
*Sugar, 5 per cent. in water.		
Salt, 3 per cent. in water.		
Methylene-blue, 1.5 : 1000.....		20
Animal charcoal.....		0.1

CHAPTER XXII

Defibrinated blood.....	25
NaCl, 0.9 per cent.....	15
*NaCl, 0.9 per cent. + $\frac{1}{10}$ per cent. saponin.....	15
*NaCl, 0.9 per cent. + $\frac{1}{10}$ per cent. saponin, 15 c.c., digested with 6 drops of 1 per cent. cholesterin.	
*NaCl, 0.9 per cent., saturated with ether.....	15
*Urea, 1 per cent.....	15
Sod. Carbonate, 2 per cent.....	15

CHAPTER XXV

**Egg-white, 1 : 100 c.c. water.	
**Defibrinated blood.....	50
**Mammalian skin.	
**Dog's intestine.	
**Dog's muscle.	

CHAPTER XXVI

Soap-bark.....	10
*Aconite, 1 per cent.....	10
**Egg-white.....	1

CHAPTER XXIX

**Milk.....	50
Rennin.....	1
**Barley Decoction, 10 per cent.....	10
**Pancreatin, 0.1 per cent.....	10
*Formaldehyd, 0.1 per cent.....	10
*Sod. Citrate, 1 per cent.....	10

APPENDIX D.—CONTENTS OF LOCKERS FOR PHARMACODYNAMIC EXERCISES

Top Shelf

- 3 semicircular stands, 5 clamps.
- 2 induction coils, 2 electrodes.
- 1 ether mask.

Second Shelf

- 1 perfusion bottle, 1 Woulff bottle.
- 1 funnel, 2 flasks—250 c.c.
- 2 tumblers, 2 beakers.
- 2 electric keys, 1 oncometer and clamp.
- 2 evaporating dishes, 2 frog boards.
- 1 mesentery board, 1 foot board.
- 1 dissecting needle, parchment, wax slides.
- sandpaper, two 25 c.c. graduates.

Third Shelf

- 1 aneurysm needle, two 10 gm. lead weights.
- 4 Mohr clamps, 3 bulldogs.
- 2 hemostats, 1 electric connection.
- 1 cork with pins, 1 knitting needle.
- 1 brass T, 2 pithing wires.
- 1 box with 2 glass Y, with 1 glass T, and 5 vessel cannulæ.
- 2 camel's hair brushes.
- 2 tracheal cannulæ, 1 large screw-clamp.
- 1 tracheal tube, 1 small screw-clamp.
- 2 heart levers.
- 4 muscle levers (2 straight, 2 elbow).
- 4 watch-glasses.
- 2 bundles ligatures, 1 suture needle.
- 2 feathers.

Wall

- two 10 c.c. pipets in $\frac{1}{10}$.
- two 10 c.c. pipets in $\frac{1}{50}$.
- 1 clinical thermometer.
- 1 thermometer, 1° to 100° C.
- 1 blood-pressure pipet.
- 1 electric signal marker.
- 1 stirring rod.
- 1 syringe, 10 c.c.
- 1 syringe, 1 c.c.
- 4 needles in bottle.
- 1 femur clamp.

Bottom Shelf

- 2 Harvard kymographs and vane.
- 2 drums; one 100-c.c. cylindric graduate.
- 1 stomach bulb and tube.
- 1 small gag, 1 large gag.
- 2 clamps (G).
- 1 mercury manometer and guide.

Drawer, Left

- 2 towels, 1 sponge, 1 set ropes.

Cupboard

- 1 saucepan, 10 test-tubes with rack and brush.
- Wooden bench.
- Bottles of saline (0.75 and 0.9 per cent.), ether, and MgSO₄.
- Artificial respiration bellows.
- 1 buret (25 c.c.), stand, clamp, and tube.

APPENDIX E.—ALPHABETIC LIST OF SOLUTIONS NEEDED FOR PHARMACODYNAMIC EXERCISES

No. of bottles.	Drugs.	Strength.	Size.
2	**Acacia.....	25 per cent.	(30 c.c.)
2	Acid, Acetic.....	5 per cent.	(200 c.c.)
2	Acid Fuchsin.....	5 per cent.	(5 c.c.)
2	Acid, Hydrochloric.....	0.5 per cent.	(10 c.c.)
6	**Acid, Hydrochloric, 0.5 per cent. in 10 per cent. Acacia.....		(10 c.c.)
2	*Acid, Hydrocyanic.....	2 per cent.	(5 c.c.)
2	*Acid, Lactic.....	0.6 per cent.	(20 c.c.)
1	Acid, Nitric.....	Conc.	(50 c.c.)
2	Aconite (Tinct.).....	10 per cent.	(15 c.c.)
2	**Aconite.....	4 per cent.	(5 c.c.)
1	**Aconitin.....	1 : 10,000	(1 c.c.)
2	Alcohol.....	95 per cent.	(15 c.c.)
2	Alcohol.....	50 per cent.	(5 c.c.)
3	Alcohol.....	25 per cent.	(50 c.c.)
2	Alcohol.....	10 per cent. in N. S.	(25 c.c.)
2	Alcohol.....	1 : 100 in N. S.	(25 c.c.)
2	Alcohol.....	1 : 1000 in N. S.	(25 c.c.)
2	*Alypin.....	1 per cent.	(1 c.c.)
2	Ammon. Chlorid.....	1 per cent.	(150 c.c.)
3	Ammonia Water.....		(10 c.c.)
2	Amyl Nitrite.....		(5 c.c.)
2	Antimonium Potas. Tartrate.....	$\frac{1}{4}$ per cent.	(50 c.c.)
2	*Antipyrin.....	2 per cent.	(25 c.c.)
3	**Apomorphin.....	1 per cent.	(10 c.c.)
2	**Apomorphin.....	1 : 1000 N. S.	(5 c.c.)
4	*Atropin Sulphate.....	1 per cent.	(10 c.c.)
4	*Atropin Sulphate.....	1 : 1000	(10 c.c.)
2	Barium Chlorid.....	1 : 1000	(5 c.c.)
3	Barium Chlorid.....	1 per cent. in N. S.	(25 c.c.)
1	*Beta-tetra-hydro-naphthylamin.....	5 per cent.	(5 c.c.)
2	**Bismuth Suspension.....		(50 c.c.)
3	*Caffein.....	1 : 100	(25 c.c.)
2	*Caffein.....	1 : 1000	(25 c.c.)
2	*Caffein.....	1 : 5000	(5 c.c.)
2	*Caffein.....	1 : 10,000	(25 c.c.)
2	Calcium Chlorid.....	10 per cent.	(5 c.c.)
2	Calcium Chlorid.....	3 per cent.	(10 c.c.)
2	Calcium Chlorid.....	1.6 per cent.	(200 c.c.)
4	Calcium Chlorid.....	1 per cent. in N. S.	(25 c.c.)
2	Calcium Chlorid., 0.15 per cent. in 0.9 per cent. NaCl.....		(200 c.c.)
2	*Camphor.....	20 per cent. in oil	(50 c.c.)
2	*Camphor.....	1 per cent. in 40 per cent. alcohol	(25 c.c.)
2	*Camphor.....	Sat'd in N. S.	(10 c.c.)
1	Capsicum Petrolatum.....		(1 c.c.)
2	Caramel.....		(10 c.c.)
6	*Cevadin.....	1 : 1000	(10 c.c.)
2	Charcoal.....		(10 gm.)
3	Chloral Hydrate.....	25 per cent.	(100 c.c.)
2	Chloral Hydrate.....	10 per cent.	(25 c.c.)

No. of bottles.	Drugs.	Strength.	Size.
2	Chloral Hydrate.....	2 per cent.	(5 c.c.)
2	Chloral Hydrate.....	1 per cent.	(5 c.c.)
2	*Chloroform.....	Sat'd in N. S.	(20 c.c.)
6	*Chloroform.....		(50 c.c.)
1	**Cholin.....	1 : 1000	(20 c.c.)
2	**Cocain Hydrochlorid.....	5 per cent.	(2 c.c.)
2	**Cocain Hydrochlorid.....	2 per cent.	(5 c.c.)
6	**Cocain Hydrochlorid.....	1 per cent.	(2 c.c.)
2	Colchicum, Fld. Ext.....		(5 c.c.)
2	Copper Sulphate.....	1 per cent.	(50 c.c.)
1	**Cotarnin.....	1 : 1000	(10 c.c.)
3	*Curare.....	$\frac{1}{2}$ per cent.	(15 c.c.)
2	*Curare.....	1 : 1000 N. S.	(5 c.c.)
3	Digitalis (Tinct.).....	10 per cent.	(10 c.c.)
2	**Digitalis.....	4 per cent.	(100 c.c.)
2	**Digitalis.....	1 per cent.	(10 c.c.)
2	*Dionin.....	10 per cent.	(1 c.c.)
6	Epinephrin.....	1 mg. tablets	(1 tube)
2	Ergot, Fld. Ext.....		(5 c.c.)
2	Ergot (Tinct.).....	10 per cent.	(10 c.c.)
2	*Ether.....	Sat'd in N. S.	(20 c.c.)
1	*Fluorescein Sol.....		(5 c.c.)
1	**Glucose.....	6 per cent.	(400 c.c.)
1	Histamin.....	Tablets	(1 mg.)
1	*Hydrastinin.....	2 per cent.	(10 c.c.)
2	Hydrastis (Tinct.).....	10 per cent.	(5 c.c.)
2	**Hydrastis.....	2 per cent.	(10 c.c.)
1	Lead Acetate Paper.		
1	*Lycopodium Suspension.....		(10 c.c.)
2	Magnesium Chlorid.....	2.1 per cent.	(200 c.c.)
2	Magnesium Sulphate.....	25 per cent.	(10 c.c.)
2	Magnesium Sulphate.....	5 per cent.	(5 c.c.)
4	Magnesium Sulphate.....	3.6 per cent. (dried)	(25 c.c.)
2	Mercuric Chlorid.....	1 : 1000	(15 c.c.)
2	*Morphin Hydrochlorid or Sulphate.....	4 per cent.	(25 c.c.)
2	*Morphin Hydrochlorid.....	1 : 1000	(5 c.c.)
1	**Muscarin.....	0.1 per cent.	(1 c.c.)
1	Nicotin.....	Undil.	(1 c.c.)
2	*Nicotin.....	1 per cent.	(5 c.c.)
2	*Nicotin.....	1 : 1000 N. S.	(5 c.c.)
2	Nitroglycerin.....	1 : 100	(5 c.c.)
4	*Nitroglycerin.....	1 : 1000	(10 c.c.)
2	*Novocain.....	1 per cent.	(1 c.c.)
2	*Ouabain.....	1 : 1000	(1 c.c.)
2	**Ouabain.....	1 : 10,000	(2 c.c.)
3	**Ouabain.....	1 : 50,000	(2 c.c.)
2	**Peptone, Witte's.....	20 per cent.	(25 c.c.)
2	*Phenol.....	1 per cent.	(100 c.c.)
3	**Physostigmin Salicylate or Sulphate.....	1 per cent.	(1 c.c.)
4	**Physostigmin Salicylate or Sulphate....	1 : 1000 N. S.	(5 c.c.)
2	*Picrotoxin.....	1 : 250	(5 c.c.)

No. of bottles.	Drugs.	Strength.	Size.
3	*Pilocarpin Hydrochl.....	1 per cent.	(5 c.c.)
2	*Pilocarpin Hydrochl.....	1 : 1000	(15 c.c.)
4	Potassium Chlorid.....	10 per cent.	(10 c.c.)
2	Potassium Chlorid.....	1 per cent.	(50 c.c.)
2	Potassium Chlorid.....	1 : 100 N. S.	(25 c.c.)
2	Potassium Chlorid.....	1 : 1000 N. S.	(25 c.c.)
2	Potassium Chlorid.....	1 : 10,000 N. S.	(25 c.c.)
2	Potassium Permanganate.....	1 per cent.	(50 c.c.)
2	Quinin Hydrochlorid.....	1 : 100 N. S.	(25 c.c.)
2	Quinin Hydrochlorid.....	1 : 1000 N. S.	(25 c.c.)
3	*Quinin Hydrochlorid.....	1 : 10,000 N. S.	(25 c.c.)
2	**Quinin-urea Hydrochl.....	1 per cent.	(1 c.c.)
6	Ringer's Solution.....		(400 c.c.)
2	Ringer's Solution.....	without Ca	(10 c.c.)
2	Ringer's Solution.....	without K	(10 c.c.)
2	Ringer's Solution.....	with CaCl ₂ , 0.8 : 1000	(10 c.c.)
2	Ringer's Solution.....	Triple strength	(10 c.c.)
2	*Saponin.....	1 : 1000 N. S.	(5 c.c.)
2	*Scopolamin Hydrobromid.....	1 : 1000	(2 c.c.)
2	Silver Nitrate.....	1 per cent.	(5 c.c.)
2	Sod. Acid Phosphate.....	2 per cent.	(200 c.c.)
3	Sodium Arsenate.....	5 per cent.	(10 c.c.)
2	Sodium Chlorid.....	Powder	(1 gm.)
2	Sodium Chlorid.....	10 per cent.	(50 c.c.)
2	Sodium Chlorid.....	5 per cent.	(500 c.c.)
2	Sodium Chlorid.....	2 per cent.	(200 c.c.)
4	*Sodium Citrate.....	5 per cent.	(25 c.c.)
2	Sodium Citrate.....	2.7 per cent.	(400 c.c.)
2	Sodium Fluorid.....	0.5 per cent.	(25 c.c.)
2	*Sodium Nitrite.....	10 per cent.	(5 c.c.)
2	*Sodium Nitrite.....	1 per cent.	(5 c.c.)
2	*Sodium Nitrite.....	0.1 per cent.	(5 c.c.)
2	Sodium Phosphate.....	2.1 per cent.	(200 c.c.)
1	*Sodium Santonin.....	5 per cent.	(25 c.c.)
2	Sodium Sulphate.....	2.5 per cent.	(400 c.c.)
2	Sodium Sulphate.....	1.9 per cent.	(200 c.c.)
1	Sodium Veronal.....	10 per cent.	(25 c.c.)
2	*Sparteïn.....	1 per cent.	(15 c.c.)
2	*Stovain.....	1 per cent.	(1 c.c.)
2	**Strophanthus.....	1 : 100	(5 c.c.)
5	**Strophanthus.....	1 : 1000	(10 c.c.)
2	*Strychnin (Sulphate or Nitrate).....	1 : 100	(5 c.c.)
6	*Strychnin (Sulphate or Nitrate).....	1 : 1000	(20 c.c.)
2	*Strychnin (Sulphate or Nitrate)...	1 : 10,000	(5 c.c.)
2	**Sugar, Cane.....	10 per cent.	(200 c.c.)
2	**Tannin.....	10 per cent.	(5 c.c.)
2	**Tannin.....	1 per cent.	(5 c.c.)
2	*Theobrom. Sod. Salic. or Acet.....	10 per cent.	(5 c.c.)
2	*Theobrom. Sod. Salic. or Acet.....	1 : 100	(25 c.c.)
2	*Theobrom. Sod. Salic. or Acet.....	1 : 1000	(25 c.c.)
2	*Theobrom. Sod. Salic. or Acet.....	1 : 10,000	(25 c.c.)

No. of bottles.	Drugs.	Strength.	Size.
1	*Theophyllin Sod. Acetate.....	10 per cent.	(10 c.c.)
2	*Tropacocain.....	1 per cent.	(1 c.c.)
1	**Tyramin.....	1 : 1000	(20 c.c.)
2	**Urea.....	1.9 per cent.	(200 c.c.)
6	*Urethane.....	10 per cent.	(2 c.c.)
2	*Veratrin.....	1 : 10,000	(1 c.c.)
6	**Veratrin.....	1 : 1,000,000	(25 c.c.)
2	Veratrum (Tinct.).....	10 per cent.	(5 c.c.)
4	Zinc Sulphate.....	1 per cent.	(50 c.c.)

TWO (2) LITER BOTTLES

Locke's Solution—Glucose free (2 bottles).

Ringer's Solution.

Sodium Chlorid, 10 per cent.; 2 per cent.; 1 per cent. (5 bottles); 0.9 per cent.; 0.75 per cent.

Tyrodé's Solution (Glucose free).

APPENDIX F.—TABULATION OF ANIMALS REQUIRED FOR DEMONSTRATIONS AND FIVE GROUPS OF STUDENTS

APPENDIX F

TABULATION OF ANIMALS

Days of Pharmacodynamic Course.	Chapter.	D = Demonstration. C = Class.	Normal Dogs.			Morphin Dogs.			Cats.			Rabbits.			Frogs.			Other Animals.	Notes.
			Total.	New.	Survive.	Total.	New.	Survive.	Total.	New.	Survive.	Turtles.	Ordinary.	Large.					
1	32	D	14	..		* Female. * Decebrated. * Female. * Rooster. * White. * = 20 gm.	
2	33 and 34	D	22	1			
3	33 and 34	D	I*	O	O	10	..			
4	35 and 36	D	I*	I	O	10	..			
5	35 and 36	D	2	I*	I	I	..	5	1		
6	37	D	2	5	3			
7	38	D	1	5	..	3 Guinea-pigs.		
8	39	D	1	6	..			
9	40	D	1	3	..	White mouse.		
10	41	D	4	..			
11	42	C	6	..			
12	43	C	0	..			
13	44	C	2	..			
14	45	C	2	..			
15	46	C	3	..			
Total animals needed.....			35			16	16	12	88	5		

* = 20 gm.

APPENDIX G.—SOLUTIONS AND MATERIALS NEEDED FOR INDIVIDUAL PHARMACODYNAMIC EXERCISES

CHAPTER XXXII.—LOCATIONS OF ACTIONS, ETC.

Groups or Demonstration.	Exercise.	Animals.	Solutions. ¹	Special apparatus.
Demonstrations:	I	7 Frogs.	Strychnin, 1 : 1000 ($\frac{1}{4}$). Acetic Acid, 5% (10). Acid Fuchsin, 5% (2). Picrotoxin, 1 : 250 (1.5). Veratrin, 1 : 10,000 ($\frac{1}{2}$). Caffein, 1 : 100 (1).	Pipet and needle. Tenaculum. Oil-bath. Strong scissors. Fine scissors. Fine forceps. Fine ligatures. Inductarium (single shocks). Aortic cannula. Perfusion bottle. Small cannula. Bell-jar. Cotton. Bell-jar and aspirator.
	II	Frog.	0.75% NaCl (200). Ether (10).	
	IV	5 Frogs.	Curare, $\frac{1}{2}$ % (2). Nicotin, 1 : 1000 (1). Tobacco (5).	
	V	Rabbit.	Cocain, 1% ($\frac{1}{4}$). Quinin-urea HCl, 1% ($\frac{1}{4}$). Tr. Aconite (10). Ethyl Chlorid (10).	Mounted bristles.
All A Groups:	I	Frog.	Strychnin, 1 : 1000 ($\frac{1}{4}$).	
	III	Frog.	HCl, 0.5% (10).	
	V	Cocain, 1% (2).	
Groups: I, A	III	HCl, 0.5% in 15% Acacia (10).	
	V	HCN, 2% (1).	
II, A	III	Alcohol, 10% ($\frac{1}{4}$).	
	V	Stovain, 1% (1).	
III, A	III	Urethane, 10% (2).	
	V	Quinin-urea, HCl 1% (1).	
IV, A	III	Morphin, 4% ($\frac{1}{4}$).	
	V	Magn. Sulph., 25% (1).	
V, A	III	Strychnin, 1 : 10,000 ($\frac{1}{4}$).	
	V	2 Frogs.	Epinephrin, 1 : 1000 (1). ² Epinephrin, 0.1% with Cocain, 1% (1).	
All B Groups:	II	Frog.		
	V	Frog.	(HCl, 0.5%, use that of A Groups.) (Cocain, 1%, use that of A Groups.)	
I, B	II	Morphin, 4% (2).	Basin.
	IV	Curare, 1 : 1000 N. S. (5). Physostigmin, 1 : 1000 N. S. (3).	
	V	Novocain, 1% (1).	
II, B	II	Alcohol, 25% (2).	
	IV	Nicotin, 1 : 1000 N. S. (5). Physostigmin, 1 : 1000 N. S. (3).	
	V	Novocain, 1% (1).	
III, B	II	Chloral, 2% (1).	
	IV	Magnes. Sulph., 5% (5). Physostigmin, 1 : 1000 N. S. (3).	
	V	Quinin-urea HCl, 1% (1).	

¹ The figures in parentheses are the cubic centimeters used in the experiment.

² Tablets.

Groups or Demonstration.	Exercise.	Animals.	Solutions.
<i>All B Groups:</i>			
IV, B	IV	Saponin, 1 : 1000 N. S. (3).
	V	Tropacocain, 1% (1).
V, B	II	Magnes. Sulph., 25% (1).
			Apomorphin, 1 : 1000 N. S. (3).
			Alypin, 1% (1).
<i>Total Animals Needed.</i> —Demonstrations: 14 frogs, 1 rabbit (s). ¹ Class Work: 22 frogs.			

CHAPTER XXXIII.—MUSCULAR CONTRACTIONS

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
<i>Demonstrations:</i>				
	III	2 Frogs.	Caffein, 1 : 10,000 N. S. (25). Caffein, 1 : 1000 N. S. (25). Quinin HCl, 1 : 10,000 N. S. (25).	Muscle lever, induction coil, and kymograph, set up for tracing as pattern.
	IV	3 Frogs.	Quinin HCl, 1 : 1000 N. S. (25). Alcohol, 1 : 100 N. S. (25).	Maximal load spring. Arrangement for Exercise IV.
	VI	Large Frog.	Perfusion bottle set up, with water. Aorta cannula. Stand and hook.
	VIII	3 Frogs.	NaCl, 10% (5). Ringer's Solution (5). Ringer's Solution without Ca (5). Ringer's Solution without K (5). Ringer's Solution, triple strength (5).	Operating instruments.
	IX	Ether, sat'd in N. S. (10).	XI, 2, see Experiment.
	X	2 Frogs.	Tannin, 1% (5). Zinc Sulphate, 1% (5). Copper Sulphate, 1% (5). Tannin, 10% (5). Epinephrin, 1 : 1000 (5).	Lung arranged as pattern.
<i>All Groups:</i>				
(A or B).				
Group I:	I	Frog.	Veratrin, 1 : 10,000 (1).	
	II	Frog.	Caffein, 1 : 10,000, 1 : 1000, 1 : 100 N. S. (25).	
Group II:	I	CaCl ₂ , 1% in N. S. (25). Sod. Citrate, 5% (25).	
	VII	Theobromin Sod. Salic., 1 : 10,000, 1 : 1000, 1 : 100 N. S. (25).	
Group III:	I	Quinin HCl, 1 : 10,000, 1 : 1000, 1 : 100 N. S. (25).	
	II	KCl, 1% (1).	
	VII	Calcium Chlorid, 1% (25). Barium Chlorid, 1% in N. S. (25).	I.e.

¹ (s) = survives.

Groups or Demonstration.	Exercise.	Animals.	Solutions.
Group IV:	I	KCl, 1 : 10,000, 1 : 1000, 1 : 100 N. S. (25).
	VII	Sod. Citrate, 5% (25). KCl, 0.1% in N. S. (25).
Group V:	I	Alcohol, 1 : 1000, 1 : 100, 1 : 10 N. S. (25).
	VII	Sod. Fluorid, 0.5% (25). Calcium Chlorid, 1% in N. S. (25).

Total Animals Needed.—Demonstration: 1 large frog, 10 ordinary frogs. Class Work: 10 frogs (half class).

CHAPTER XXXIV.—SMOOTH MUSCLE

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
<i>Demonstrations:</i>	I	Decerebrated Rabbit.	NaCl ($\frac{1}{2}$).	Operating instruments.
			Physostigmin, 1% (1).	Board.
			Barium Chlorid, 1% (4).	Injection buret, connections, and clamp.
			Atropin, 1% (4).	Hypodermic syringe.
			Nicotin, 1% (1).	Tracheal cannula.
			Normal saline (200).	Vein cannula.
			Pilocarpin, 1% (15).	Respiration bellows.
			Pituitary Solution (1.5).	Bell-jar.
				CO ₂ apparatus.
				Induction coil.
	V	Female Rabbit.	Warm Tyrode Solution (2000).	Ligatures.
			Warm Normal Saline, 0.9 (3000).	Water-bath at 40° C.
			Oxygen.	Aortic cannula.
		Sheep's Carotid.		Dish and rods for defibrinating.
				Lever, etc., set up as pattern.
				Water-bath at 40° C
				Air current.
All Groups:	VI to IX	2 Cylinders of Tyrode Solution.	
Group I:	VI,	Sod. Nitrite, 10% (1).	
	VIII,	Epinephrin, 1 : 10,000 ($\frac{1}{2}$).	
	IX	Pilocarpin, 1 : 1000 (1).	
Group II:	VII	Atropin, 1 : 1000 (1).	
	VI	Barium Chlorid, 10% (2).	
	VI and VIII	Sod. Sulphate, 1.9% (200).	
	VII	Atropin, 1% (1).	
	VIII	Pituitary Solution, ($\frac{1}{2}$).	
Group III:	IX	Sod. Citrate, 2.7% (200).	
	VI	Epinephrin, 1 : 10,000 ($\frac{1}{2}$).	
	VII	Pilocarpin, 1% (1).	
	VIII	Atropin, 1% (1).	
Group IV:	IX	Magnesium Chlorid, 2.1% (200).	
	VI	Quinin HCl, 1% (1).	
	VII	Barium Chlorid, 1% (5).	
	VIII	Nicotin, 1% (1).	
	IX	Atropin, 1% (1).	
			Calc. Chlorid, 0.15% in 0.9 NaCl (200).	
			F. E. Ergot (1).	
			Tr. Digitalis (1).	

Groups or Demonstration.	Exercise.	Animals.	Solutions.
Group V:	VI	Barium Chlorid, 1% (5).
			Atropin, 1% (1).
	VII	NaCl, 2% (200).
	VIII	Tr. Hydrastis (1).
	IX	Physostigmin, 1% (1).

Total Animals Needed.—Demonstrations: Decerebrated rabbit (f).¹ Class Work: Female rabbit (f) (Half class).

CHAPTER XXXV.—PERFUSION EXPERIMENTS

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
<i>Demonstrations:</i>	I	White Rabbit.	Nicotin, 1% (1).	Hypodermic syringe.
	II	Rooster.	F. E. Ergot (5).	
	IV	Large Frog.	Sod. Nitrite, 0.1% (1).	Mariotte bottle with connection and clamp, on stand.
			Epinephrin, 1 : 5,000,000 (1).	
			Digitalis, 1 : 100 (1).	
			Ringer's Fld. (500).	Forceps, coarse and fine.
				Strong and fine scissors.
				Fine ligatures.
				Frog board.
				Drop-counter.
				Aortic cannula.
				Vein cannula.
				Perfusion bulb.
				Operating instruments.
				5 cannulae (renal artery); carotid and femoral cannula.
				Dish, rods, and strainer for blood.
All Groups:	VII	NaCl, 1% (1000). (Except Group I.)	Perfusion stand with connections.
	IX	Amyl Nitrite (1).	Oncometer bulbs.
Group I:	VII	NaCl, 2% (2000).	
Group II:	VII	NaCl, 5% (500).	
			Calc. Chlorid, 1.6% (200).	
			Sod. Citrate, 2.75% (500).	
Group III:	VII	Epinephrin, 1 : 1000 (1).	
			HCN, 2% (2).	
			Tr. Digitalis (1).	
			Chloral, 10% (1).	
Group IV:	VII	Barium chlorid, 1% (5).	
			Defibrinated blood (200).	
			HCN, 2% (2).	
			Caffein, 1% (2).	
			Tr. Digitalis (1).	
Group V:	VII	Epinephrin, 1 : 10,000 (5).	
			Sod. Nitrite, 1 : 100 (5).	
			Digitalis, 1 : 100 (5).	
			Chloral, 1% (5).	
			Barium Chlorid, 1 : 1000 (5).	
Groups II, III:	IX	Sphygmomanometer.
Groups IV, V:	IX	Plethysmograph.

Total Animals Needed.—Demonstrations: White rabbit (s); Rooster (s); Large frog. Class Work: 2 Morphinized dogs (f) (Half class).

¹ Fatal.

CHAPTER XXXVI.—EXCISED HEARTS

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
<i>Demonstrations:</i>	I	Morphin- ized dog.	Ether, 100. Warm Locke Fluid, 3000. Oxygen. Strychnin, 1 : 5000 (5). Caffein, 1 : 5000 (5). Chloroform, sat'd in N. S. (5). Epinephrin, 1 : 10,000 (5). KCl, 1 : 100 (5). Camphor, sat'd in N. S. (5). Digitalis, 1 : 100 (5). Ringer's Solution (50). Ca-free Ringer (10). Ringer with Calcium Chlo- rid 0.8 : 1000 (10). Aconitin, 1 : 10,000 (1). Potassium Chlorid, 10% (0.5). Strychnin Sulph., 1 : 1000 (1). Strychnin Sulph., 1 : 100 (1). Caffein, 1 : 100 (1). Epinephrin, 1 : 10,000 (1). Ouabain, 1 : 50,000 (1.5). Tr. Digitalis (1). " diluted $\frac{1}{2}$ (1.2).	Langendorff appa- ratus. Injection buret and funnel. Operating instru- ments. Bone-forceps. Ligatures. Cannulæ for carotid, femoral, trachea, and aorta. Dish, rods, funnel, and strainer for blood. Hypodermic syringe. Straub-Fuehner can- nula, etc., Ex. II, 2.
	III	6 Frogs of abt. 20 gm.		Pithing needle.
	V	Frog.		Pipet and needle.
	VI	Turtle.	Ringer's Solution (2000).	Heart lever and stand set up for pattern.
	VII	Turtle.	Pilocarpin HCl, 0.5% (1). Atropin Sulph., 0.1% (1).	Perfusion bottle and cannulæ, set up for pattern.
	VIII	Frog.	Muscarin or physostigmin, 0.1% (1).	Hammer. Bone-forceps. Saw. Turtle-lever and drum. Induction coil.
<i>Class Work:</i>	IV	Frog.	Urethane, 10% (2).	
All groups:	VI	Turtle.	Ringer's Solution (250).	
Groups I, II:	IV	Digitalis, Tr. (1).	
Groups III, IV	IV	Aconite, 4% (5).	
Groups I, II, III, IV:	VI	Epinephrin, 1 : 100,000 (1).	
Group I:	VI	Antipyrin, 1% (2.5).	
	IX	Alcohol (10).	
Group II:	VI	Tr. Aconite (2).	
	IX	Strychnin, 1 : 1000 (2).	
			Caffein, 1 : 100 (2).	
Group III:	VI	Alcohol (7).	
	IX	Ouabain, 1 : 10,000 (2).	

Groups or Demonstration.	Exercise.	Animals.	Solutions.
Group IV:	VI, IX	KCl, 10% (7).
	IX	Epinephrin, 0.1% (2).
Group V:	IV	Chloroform in N. S. (10).
		Ether in N. S. (10).
	VI	Tr. Digitalis (0.1).
		KCl, 10% (1).
	IX	Calc. Chlorid, 10% (2).
		Epinephrin, 0.1% (2).

Total Animals Needed.—Demonstrations: Morphinized dog (f); 9 frogs (1 large, 2 medium, 6 of about 20 gm.); 2 turtles. Class Work: 5 frogs; 5 turtles (for half class).

CHAPTER XXXVII.—PUPILS, ETC.

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
<i>Demonstrations:</i>	I	Morphinized dog.	Ether (200). Atropin, 1 : 1000 (2). Physostigmin, 1 : 1000 (2).	Board. Operating instruments. Induction coil. Injection syringe. Vein cannula. Rubber rings (20). Hypodermic syringe.
	III	
	V	2 Cats or Rabbits.	Pilocarpin, 1% (3). Atropin, 1% (10).	
	VI	Dilute acetic acid (200).	
	VII	Rabbit.	Epinephrin, 1 : 10,000 (2). Pilocarpin, 1 : 1000 (2). Histamin, 1 : 10,000 (1).	Board. Tracheal cannula. Motor bellows. T-piece. Pleural cannula. Tambour. Kymograph. Jugular cannula. Pithing rod. Pipet.
	VIII	Guinea-pig.	Tyrode solution (Glucose free) (500). Peptone, 1% in Tyrode (250).	Pulmonary artery cannula. Perfusion bottles, connections, and stand.
	X	2 Sensitized guinea-pigs.	Horse serum (2). Chloroform (20).	
	XII	Calcium cat. Normal cat.	Dionin, 10% (½).	

Class Work, A Groups:

I	II	Cat.	Atropin, 0.1% (½).
			Pilocarpin, 1% (½).
			Physostigmin, 1% (½).
II	II	Cat.	Physostigmin, 1% (½).
III	II	Cat.	Pilocarpin, 1% (½).
IV	II	Cat.	Cocain, 1% (½).
V	II	Cat.	Dionin, 10% (½).

Class Work, B Groups:

All B Groups:	III	Frog.	Pilocarpin, 1% (½).
Groups I, III to V:	Physostigmin, 1% (½).
Group II	Atropin, ½% (½).
Group III	Nicotin, 0.1% (½).
Group IV	Cocain, 1% (½).
Group V	..	2 Frogs.	Epinephrin, 1 : 10,000 (½).
			Epinephrin, 1 : 1000 (1).

Total Animals Needed.—Demonstrations: Morphinized dog (f); rabbit (f); 2 cats or rabbits (s); 3 guinea-pigs (f); 2 cats (s). Class Work: 5 cats (s); 6 frogs.

CHAPTER XXXVIII.—ABSORPTION, ETC.

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
<i>Demonstrations:</i>	I	Morphinized dog.	Ether (200). Epinephrin, 1 : 1000 (5). Strychnin, 1 : 100 (3).	Mercury manometer and connections. Double kymograph. Carotid and tracheal cannulae. 2 Femoral cannulae. Hypodermic syringe. Injection buret. Pipet.
	II	2 Rabbits or cats (sick).	Nicotin ($\frac{1}{2}$). HCN, 2% (5).	
	III	Rat, cat, or guinea-pig.	Bell-jar with coal-gas.
	VII	Rabbit or cat.	Lead acetate paper.	H ₂ S apparatus. Rectal tube.
	VIII	Rabbit with iodid, morphin, and calomel. Rabbit with calomel.	Fluorescein Solution (1).	
<i>Class Work:</i>				
Group I	IV	2 Rabbits.	Strychnin, 1 : 1000 (5).	
	XVI	Cat or dog.	Copper Sulph., 1% (50).	
	XVII	Dog.	Morphin, 4% (3). Apomorphin, 1% (1).	
Group II	V	2 Cats.	Chloral, 10% (15).	
	XVI	Cat or dog.	Zinc Sulph., 1% (50).	
	XVII	Cat or dog.	Morphin, 4% (3). Zinc Sulphate, 1% (50).	
Group III	VI	2 Cats.	Strychnin, 1 : 1000 (6). Acacia, 25% (30).	
	XVIII	Cat.	Bismuth Suspension (50). Zinc Sulph., 1% (25).	
	XVI	Cat or dog.	Antim. Potas. Tart., $\frac{1}{4}$ % (40).	
Group IV	XI	Dog and rabbit.	Ammonia vapor.	
Group V	XIV	1 Dog. 2 Cats. 1 Rabbit.	Atropin, 1% (10). Apomorphin, 1% (20).	

Total Animals Needed.—Demonstrations: Morphinized dog (f); 2 rabbits or cats (f); 2 rabbits or cats (s); 2 rabbits (s). Class Work: (s) 3 rabbits; 5 cats; 4 dogs or cats; 3 dogs; (f): 1 rabbit; 1 cat.

CHAPTER XXXIX.—TEMPERATURE, ETC.

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
<i>Demonstrations:</i>	XI	Rabbit.	HCl, 1% (300).	Stomach-tube. Vein cannula. Buret and stand. Hypodermic syringe.
	XIII	White mouse.	Morphin, 1 : 1000 ($\frac{1}{2}$).	
	XV	Rabbit.	Magn. Sulph., 25% (15). Calc. Chlorid, 3% (10).	Operating instruments.
	XX	Uranium rabbit.		
	XXIII	Morphinized dog.	Ether (200). Acetic acid, 5% (10). Nitric acid (50).	Blood-pressure. Pipet. Injection buret.
	XXIV	Sod. Arsenate, 5% (10).	

Groups or Demonstration.	Exercise.	Animals.	Solutions.
<i>Class Work:</i>			
Group I	..	4 Cats.	Chloral, 2.5% (200). Caffein, 1% (3). Strychnin, 0.1% (½).
Group II	..	Rabbit, dog, and cat.	Morphin, 4% (10). F. E. Colchicum (5). Mercuric Chlor., 1 : 1000 (15). Sod. Arsenate, 1% (3).
Group III	..	2 Cats, 1 dog, and 1 rabbit.	Sod. Santonin, 5% (25). Ext. Cannabis (0.5) in capsules. Alcohol, 25% (25). Zinc Sulphate, 1% (25).
Group IV	..	2 Cats, 1 rabbit or cat. 1 Rabbit.	Cocain, 5% (2). Beta-tetra-hydro-naphthyl-amin, 5% (3). Alcohol, 25% (25). Caffein, 1% (15).
Group V	..	3 Rabbits.	Witte Peptone, 20% (25). Antipyrin, 2% (25).

Total Animals Needed.—Demonstrations: Morphinized dog (f); rabbits, 3 (f); white mouse (s). Class Work: (f) 15 cats; 2 rabbits; 1 dog; 1 rabbit or cat; (s) 4 cats; 4 rabbits; 1 dog.

CHAPTER XL.—CONVULSANTS, ETC.

Groups or Demonstration.	Exercise.	Animals.	Solutions.
<i>Class Work:</i>			
Group I	..	1 Cat or rabbit. 1 Bromid cat or rabbit.	Camphor, 20% in oil (60). Chloroform (10).
Groups II to V	..	2 Cats each.	Strychnin, 1 : 1000 (5).
Group III	Chloral, 2.5% (30).
Group IV	Potas. Permanganate, 1% (50).
Group V	Charcoal (25). Hydrocyanic acid, 1 : 1000 (10). Potas. Permang., 1% (50).

Total Animals Needed.—Class Work: 2 rabbits or cats (f); 4 cats (f); 4 cats (s).

CHAPTER XLI.—RESPIRATION

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
<i>Demonstrations:</i>	I	2 Rabbits.	Morphin, 1 : 1000 (3). Morphin, 4% (3). Camphor, 20% in oil (1.5). Caffein, 1% (3).	Tennis-ball mask. 2 Rabbit boards. Tennis-ball mask. Hot-water bottles. Tennis-ball mask.
Groups I, II, and III	..	Rabbit.	
Group I	IV	Capsicum Petrolatum (1). Chloral, 2.5% (50). Caffein, 1% (3). Alcohol, 50% (3). Strychnin, 0.1% (3). Atropin, 0.1% (3).	Bell.
Group II	V	Ammonium blowing bottle. Morphin, 1 : 1000 (2). Morphin, 4% (2). Nicotin, 0.1% (2).	
Group III	VI		
Groups IV, V	VII, VIII	Morphinized dog.		

Groups or Demonstration.	Exercise.	Animals.	Solutions.
Group IV	VII	Lactic Acid, 0.6% (20). Caffein, 1% (20). Camphor, 1% in 40% Alcohol (10). Strychnin, 0.1% (10).
Group V	VIII	Ammonia in blowing bottle. Ammon. Chlorid, 1% (150). Strychnin, 0.1% (10).

Total Animals Needed.—5 Rabbits (s); 2 morphinized dogs (f).

CHAPTER XLII.—ANESTHESIA

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
Group IV	..	2 Rabbits.		
Groups I, II, III, and V	..	Dogs.	Epinephrin, 1 : 1000 (1). Warm N. S. (500). Chloroform in blowing bottle. Cocain, 2% (5). Nitrous oxid. Chloroform (10). Morphin, 4% ($\frac{1}{2}$). Ethyl chlorid (2). Ether in blow-bottle. Morphin, 4% ($\frac{1}{2}$). Scopolamin, 1 : 1000 (2). Chloroform (25). Ether, sat'd in N. S. (25).	
Group IV, A	I		
Group IV, B	II		Rectal catheter.
Groups I, II, and V	III, IV		
Groups II, V	V		
Groups II, III, and V		Oncometer.
Group V		
Group III	VI	Scopolamin, 1% (2). Curare, $\frac{1}{2}$ % (15). Phenol, 1% (100). Epinephrin, 1 : 10,000 (200).	Compressed air or oxygen. Apparatus for insufflation, with ether and chloroform. Catheter. Cardioplethysmogram. Saw. Cautery.

Total Animals Needed.—2 Rabbits (s); 4 dogs (f).

CHAPTER XLIII.—VASOMOTOR DRUGS

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
All Groups:	..	Morphinized dog. (Smallest dog for Group II)	Epinephrin, 1 : 10,000 (10).	
Group I	Stephen Hale manometer.
Groups II, III	Oncometers.
Group IV	Cardioplethysmogram.
Group V	Vasomotor perfusion.
Groups:				
I, II	Amyl Nitrite (2).	
I, IV, V	Nitroglycerin, 1 : 1000 (10).	
I, III, IV, V	Strophanthus, 1 : 100 (1).	
II, III, IV, V	Strychnin, 1 : 1000 (1).	
II, IV	Pituitary Sol'n (1).	
II	Ergot, 25% (10).	
II	Tyramin, 1 : 1000 (20).	
II	Histamin, 1 : 10,000 (2).	

Groups or Demonstration.	Exercise.	Animals.	Solutions.
Groups:			
II	Cholin, 1 : 1000 (20).
II	Cotarnin, 1 : 100 (10).
II	Hydrastis, 2% (10).
II	Hydrastinin, 1 : 100 (5).
II	Nicotin, 0.1% (5).
III	Sod. Nitrite, 10% (5).
III	Alcohol, 25% (50).
III	Veronal, Sodium, 10% (25).
III	Peptone, 10% (50).
III	Ammonia in blow-bottle.
IV	Phenol, 1% (50).
IV	Chloral, 10% (50).
IV	Arsenate Sodium, 5% (50).
V	Chloroform (10).
V	Caffein, 1% (10).
V	Cevadin, 1 : 1000 (1).
V	Atropin, 1 : 1000 (1).

Total Animals Needed.—5 Morphinized dogs (f).

CHAPTER XLIV.—CHANGES IN HEART-RATE, ETC.

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
All Groups:	..	Morphinized dog.		
Group I	Cardiomyogram.
Group II	Cardioplethysmogram.
Groups I, II, III, IV			Cevadin, 1 : 1000 (1).	
Groups I, V	Strophanthus, 1 : 100 (5).	
Group II	Sparteïn, 1 : 100 (10).	
Group II	Pilocarpin, 1 : 100 (2).	
Group II	Digitalis, 5 : 100 (100).	
Group III	Ouabain, 1 : 1000 (1).	
Group IV	Atropin, 1 : 1000 (8).	
Group IV	Barium chlorid, 1 : 100 (30).	
Group V	Nitroglycerin, 1 : 100 (1).	
Group V	Epinephrin, 1 : 1000 (1).	

Total Animals Needed.—5 Morphinized dogs (f).

CHAPTER XLV.—MYOCARDIAL DEPRESSANTS AND TONICS

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
All Groups:	..	Morphinized dog.		
Group III	Cardiomyogram.
Group I	Cardioplethysmogram.
Group I	Aconite, 10% (15).	
Group I	Phenol, 1% (75).	
Group I, V	Veratrum, 10% (1).	
Group I, III	Chloroform (10).	
Group II, IV	Nitroglycerin, 1% (2).	
Group II, IV	Epinephrin, 1 : 1000 (1).	
Group II, IV	Ergot, 25% (10).	
Group II	Barium Chlorid, 1% (25).	
Group III	Caffeïn, 1% (25).	
Group III	Sparteïn, 1% (15).	
Group III	Digitalis, 5% (100).	
Group IV, V	Strophanthus, 1% (5).	
Group V	Strychnin, 1 : 1000 (1).	
Group V	Potassium chlorid, 1% (50).	
Group V	Camphor, 1% in 40% Alcohol (25).	

Total Animals Needed.—5 Morphinized dogs (f).

CHAPTER XLVI.—DIURESIS; CARDIAC LESIONS

Groups or Demonstration.	Exercise.	Animals.	Solutions.	Special apparatus.
All Groups:	..	Morphinized dog.	Warm Saline (500). Strophanthus, 1 : 1000 (10).	
Group I	Cardioplethysmogram.
Group V	Cardiomyogram.
Group I	Sod. Sulphate, 2.5% (400) (dried).	
Groups I, V	Epinephrin, 1 : 1000 (1).	
Group I	Sparteïn, 1% (10).	
Groups II, III, V	NaCl, 1% (25).	
Groups II, III, V	MgSO ₄ , 3.6% dried, (25).	
Group II	NaCl, 1% (400).	
Group II	Theobromin Sod. Salic., 10% (5).	
Group III	NaCl, 10% (40).	
Group III	Theophyllin, Sod. Acet., 10% (10).	
Group III	Alcohol, 95% (15).	
Group IV	Glucose, 6% (400).	
Group IV	Amyl Nitrite (5).	
Group IV	Caffeïn, 1% (15).	
Group V	Locke's Solution (no Glucose) (400).	
Group V	Pituitary Solution (1). Lycopodium Suspension (10).	

Total Animals Needed.—5 Morphinized dogs (f).

APPENDIX H.—DOSES FOR ANIMALS

The drugs are arranged alphabetically; in the case of salts, by the more important ion. In the case of crude drugs the dose refers to fluid preparations. The "just fatal" doses have generally been worked out with considerable accuracy, but may vary somewhat with different samples of the poison and with each lot of animals. The doses marked with an asterisk (*) have been confirmed by the author; the others were compiled from pharmacologic literature.

Doses of drugs not contained in this list may be ascertained by consulting the original papers cited in the Manual of Pharmacology.

M. F. D. = minimum fatal dose (average).

It is convenient to remember that a dose of 1 mg. per kg. corresponds to about 0.05 gm. or 1 grain for an adult man.

Abrin.

M. F. D.: *Rabbit*, vein, per kg., 0.01 mg.

Absinth.

Epileptic Convulsions: *Dog*, per kg., 0.03 to 0.05 c.c. of Essence (Ossipow, 1914, Ref. Zbl. Bioch. Bioph., 17, 393).

Acetanilid.

Urine: *Man*, 0.2 gm. (Chap. 15, IX).*

Toxic Dose: *Dog*, stomach, per kg., 0.7 gm.: cyanosis and methemoglobinemia, fatal in nine hours. *Rabbit*, stomach, per kg., 0.2 gm.: slowed heart and respiration; paralysis of legs; recovery in three hours.*

Acetate, Sodium.

Urine: *Man*, 10 gm.: Alkaline (Chap. 15, VI).*

M. F. D. (usual): *Dog*, vein, per kg., 3 gm.

Not dangerous: *Dog*, vein, per kg., 35 c.c. of 1.94 per cent., crystals.*¹

Acetphenetidin.

Urine: *Man*, 0.3 gm. (Chap. 15, IX).*

Acid, Acetic.

Fatal: *Dog*, stomach, per kg., 0.3 gm.

Reflex: *Frog*, 5 per cent. (Chap. 32, III).*

¹ For fatal dose of a series of Sodium Salts, see Sabbatani.

Acid, Hydrochloric.

Acidosis: *Rabbit*, stomach, per kg., 1 gm. (100 c.c. of 1 per cent.): slowed heart and respiration, ascending paralysis, convulsions, death in twelve to forty-five minutes (Chap. 39, C).* *Guinea-pig*, rectum, 10 to 50 c.c. of 1 per cent.: slowed heart and respiration, convulsions, fall of temperature, death by respiratory failure in twelve to forty-five minutes, early rigor.*

Acid, Lactic.

Medullary Stimulation: *Dog*, vein, per kg., 2 c.c. of 0.6 per cent. (Chap. 41, VII, 1).*

Acid Phosphate, Sodium.

Acidosis: *Mammals*, vein, 10 per cent. (Spiro).

Aconite.

Fatal Dose: *Dog*, hypodermic, per kg., 40 mg.: nausea, inco-ordinated movements, irregular heart and slowed and irregular respiration, convulsions in twenty-five minutes, death in thirty-four minutes.*

M. F. D.: *Guinea-pig*, hypodermic, per gm., 0.04 mg. (Chap. 36, III, 7).

Cardiac Arrest: *Mammals*, vein, per kg., 100 mg., 1 c.c. of 10 per cent.) (Chap. 45, I, 5).*

Therapeutic Dose: *Mammals*, vein, per kg., 5 mg. ($\frac{1}{20}$ c.c. of 10 per cent.) (Chap. 45, I, 1).*

Heart tracing: *Frog*, lymph-sac, 0.5 c.c. of 4 per cent. (Chap. 36, IV, 3).*

Aconitin (Crystals).

M. F. D.: *Dog*, hypodermic, per kg., 0.1 mg. *Rabbit*, hypodermic, per kg., 0.5 mg.

Guinea-pig, hypodermic, per kg., 0.06 mg. (Merck's, 0.05 mg., Engelhardt).

Pigeon, hypodermic, per kg., 0.22 mg. *Frog*, hypodermic per gm., 0.016 mg.

Acrolein.

M. F. D.: *Mammals*, stomach, per kg., 0.15-0.2 gm.

Adalin.

Hypnotic: *Dog*, stomach, per kg., 0.25 gm. (Gensler, 1915).

Adrenalin. See *Epinephrin*.**Albomose.** See *Peptone, Witte's*.**Alcohol, Amyl.**

Fall Blood-pressure: *Dog*, vein, per kg., 5 c.c. of 2 per cent. (Salant, 1909).

M. F. D.: *Rabbit*, stomach, per kg., 1.7 to 2.0 gm.

Narcotic: *Rabbit*, stomach, per kg., 0.8-1.25 gm.

Alcohol, Butyl.

M. F. D.: *Rabbit*, stomach, per kg., 2.1-2.5 gm.

Narcotic: *Rabbit*, stomach, per kg., 1.0-1.5 gm.

Alcohol, Ethyl.

Ergograph: *Man*, 20 to 40 c.c. of 20 per cent. (Chap. 33, V).

Ordinary Dose: *Mammals*, vein, per kg., 1 c.c. (4 c.c. of 25 per cent.; this concentration does not precipitate blood) (Chap. 43, III, 4).*

M. F. D.: *Rabbit*, stomach, per kg., 6.25-7.25 gm. *Cat*, peritoneum, per kg., 8 c.c.

Paralytic Dose: *Cat* or *Guinea-pig*, stomach or peritoneum, per kg., 4 c.c. (16 c.c. of 25 per cent.) (Chap. 39, XVI).* (Details, Pilcher, 1912, Jour. Pharmacol., 3, 267.) *Rabbit*, stomach, per kg., 5 c.c. (10 c.c. of 50 per cent.).* (Antipyretic,

Antidote) stomach, per kg., 2.5 to 4 gm.: narcotic; recovery in one to two hours; 4.5 to 6 gm.: narcotic; recovery in six to ten hours.

Respiratory Stimulation: *Rabbit*, hypodermic, per kg., 0.5 c.c. (1 c.c. of 50 per cent.) (Chap. 41, V, 2).*

Narcotic Dose: *Frogs*, lymph-sac, 2 c.c. of 25 per cent. (Chap. 32, II, 3).*

Reflexes: *Frogs*, lymph-sac, 0.5 c.c. of 10 per cent. (Chap. 32, III, 2).*

Alcohol, Methyl.

M. F. D.: *Rabbit*, stomach, per kg., 7-9 gm.

Narcotic: *Rabbit*, stomach, per kg., 3.2-5.5 gm. *Dog*, stomach, per kg., 4 c.c. (sleep lasting several days).

Aleuron.

Exudate: *Mammals*, pleural or hypodermic, 10 c.c. of 5 per cent. suspension in 3 per cent. starch paste (Chap. 37, XVI).

Aloin.

Nephritis: *Rabbit*, hypodermic, per kg., 2 c.c. of 5 per cent., warmed (Chap. 39, XXI).

Dog, hypodermic, per kg., 2 c.c. of 2 per cent. (MacNider, 1912).

Alum.

Retardation Intestinal Absorption: 0.25-0.5 per cent.

Aluminum Salts (Calculated as Metal).

M. F. D.: *Dog*, hypodermic, per kg., 130 mg.; ditto, *Cat*, 150 mg.; ditto, *Rabbit*, 160 mg. *Frog*, lymph-sac, 12-16 mg.

Ammonium Carbonate.

Emetic: *Dog*, stomach, 20 c.c. of 5 per cent. (Chap. 38, XVI).*

Convulsions: *Rabbit*, hypodermic, per kg., 0.4 gm. *Frog*, lymph-sac, 25 c.c. of 1 per cent. (Chap. 32, I, 9).*

Ammonium Chlorid.

Medullary Stimulation: *Mammals*, vein, per kg., 0.15 gm. (15 c.c. of 1 per cent.) (Chap. 41, VIII, 2).*

Anesthesin.

M. F. D.: *Dog*, vein, per kg., 0.4 gm.; peritoneum, per kg., 0.75 gm.; ditto, *Cat* or *Guinea-pig*, 0.9 gm. *Rabbit*, stomach, per kg., 1.15 gm.

Anilin.

Toxic: *Frog*, 2 drops in mouth.*

Antimonium Potassium Tartrate.

Emetic: *Dog* or *Cat*, stomach, 0.003 to 0.1 gm. (Chap. 38, XVI).* (Details, Pittenger, 1913.)

Fatal: *Rabbit*, vein, per kg., 0.15 (6 c.c. of 2.5 per cent.), fatal in twenty-four hours.

Antipyrin.

Urine: *Man*, 0.3 gm. (Chap. 15, X).*

Antipyretic: *Rabbit* or *Cat*, hypodermic, per kg., 0.1 gm. (1 c.c. of 10 per cent.) (Chap. 39, VI).* *Rabbit* or *Cat*, stomach, 0.5 gm. (Gottlieb, 1890).

Ordinary Dose: *Mammals*, vein, per kg., 0.1 gm. (1 c.c. of 10 per cent.) (Chap. 45, I, 2).*

M. F. D.: *White Mice*, hypodermic, per gm., 1 mg. (Hale, 1910).

Apocodein Hydrochlorid.

Nerve Paralysis: *Mammals*, vein, per kg., 40 to 50 mg. (as 1 per cent.). Local, 1 per cent. Perfusion, inject 2 c.c. of 1 per cent.

Apocynum.

Emetic: *Dog*, stomach, per kg., 0.2 gm.*

Cardiovascular: *Dog*, hypodermic, per kg., 0.35 gm.*

M. F. D.: *Frog*, lymph-sac, per gm., 0.05 mg.

Apomorphin Hydrochlorid.

Emetic: *Dog*, hypodermic, per kg., 1 mg. (0.1 c.c. of 1 per cent.); effective in two and one-half to ten minutes (Chap. 38, XIV)*; just effective, 0.2 mg. per kg. (Eggleston and Hatcher, 1912; also with other methods of administration). *Cat*, much higher.

Hypnotic: *Dog*, hypodermic, per kg., 0.04 mg. (often unsuccessful).*

Excitant: *Rabbit*, hypodermic, 10 mg.*

M. F. D.: *White Mice*, per gm., 0.4 mg. (Hale, 1910).

Arsenate, Sodium.

Cardiovascular: *Mammals*, vein, per kg., 50 mg. (1 c.c. of 5 per cent.) (Chap. 43, IV, 10).*

Nephritis: *Rabbit*, hypodermic, per kg., 10 to 35 mg. (1-3.5 c.c. of 1 per cent.) (Chap. 39, XXI).* *Dog*, hypodermic, per kg., 1-20 mg. (MacNider, 1912).

Enteritis: *Rabbit*, hypodermic, per kg., 50 mg. (1 c.c. of 5 per cent.) (Chap. 39, XVIII, 3).

Fatal: *Rabbit*, hypodermic, 5 c.c. of 5 per cent.*

Arsenic Acid.

M. F. D.: *Rabbit*, hypodermic, per kg., 12.4 mg.; fatal in two and one-half days (Kionka, 1911).

Arsenic Trioxid.

M. F. D.: *Rabbit*, hypodermic, per kg., 8.33 mg.; fatal in four days (Kionka, 1911).

Arsenite Potassium, Liqueur (Fowler's Solution).

Effects: *Rabbit*, stomach, 0.6 c.c. per kg., may be fatal inside of twelve hours; 1.5 c.c. may be survived. *Dog*, 1 c.c. per kg., vomiting, may be fatal; 3.5 c.c. may be survived; if vomiting is prevented by morphin, 0.5 to 1 c.c. may be fatal inside of twelve hours.

Aspidospermin.

Respiratory Stimulation: *Dog*, hypodermic or vein, per kg., 2.5 to 8 mg.

Atoxyl.

Fatal, *Dog*, hypodermic, per kg., 20 mg. (Details, Dietrich, 1910, Merck's Rep., 24, 117.)

Atropin Sulphate.¹

M. F. D.: *Rabbit*, per kg.: stomach, 1.4-1.5 gm.; hypodermic, 0.5-0.75 gm.; vein, 0.07-0.075 gm. *Dog*, per kg., hypodermic, 0.14-0.4 gm.; vein, 0.06-0.07 gm. *Cat*, per kg.: hypodermic, 0.03 gm. *Guinea-pig*, per kg.: hypodermic, 0.6 gm.; vein, 0.085 gm. *Rat*, per kg.: hypodermic, 2.5 gm.

¹ Doses for different animals: Cloetta, 1905; Heffter, 1911; Wilberg, 1914.

Atropin Sulphate (*Continued*).

Mydriatic: *Cat*, per kg.: stomach, 0.5 mg.; hypodermic, 0.04 mg.; vein, 0.02 mg.; rectal, 0.7 mg. (Hatcher and Eggleston, 1914).

Respiratory Stimulant: *Rabbit*, hypodermic, per kg., 1 mg. (1 c.c. of 1 : 1000) (Chap. 41, V).*

Antagonist to Pilocarpin (intestines and bronchi, etc.): *Rabbit*, vein, per kg., 1-2 mg. (1-2 c.c. of 1 : 1000) (Chap. 34, I, 10; Chap. 37, VII).*

Antagonist to Cholin: *Mammals*, vein, per kg., 1 mg. (Chap. 43, II, 8).*

Vagus Paralysis: *Dog*, vein, per kg., 0.05 mg. $\frac{1}{20}$ c.c. of 1 : 1000) (Chap. 44, IV, 4).* (Details, Sollmann and Pilcher, 1914.)

Successive Effects: *Dog*, hypodermic, per kg., 0.2 mg., not toxic; effect on pupil and heart (*); 10 mg., vomiting (*); 40 to 80 mg., severely toxic, but usually not fatal.* M. F. D. lies between 20 and 400 mg. *Cat*, hypodermic, mg. per kg.: 0.02, no effect on pupil; 0.04, good dilatation; 0.05 just paralyzes vagi. *Frog*, lymph-sac, 1 mg., little effect; 2 mg. motor depression with recovery; 10 mg. per 20 gm., fatal; 50 mg., ordinary dose; 100 mg., fatal (*).

Barium Carbonate.

Fatal: *Dog*, stomach, 4 gm.

Barium Chlorid.

Cardiovascular: *Mammals*, vein, per kg., 20 mg. (2 c.c. of 1 per cent.) (Chap. 44, IV, 5).*

Peristalsis: *Rabbit*, vein, per kg., 10 mg. (1 c.c. of 1 per cent.) (Chap. 34, I, 11).*

Benzoic Acid.

Fatal: *Frog*, lymph-sac, per gm., 2.5 mg. (three hours) (Impens).

Berberin.

Cardiovascular: *Mammals*, vein, per kg., 1-5 mg. (Chap. 43, I, 15)* (Williams, 1908).

Vagus Depression: *Mammals*, vein, per kg., 2-20 mg. (Marfori, 1890).

Vagus Paralysis: *Frog*, lymph-sac, 10 mg. (Marfori, 1890).

Beta-tetra-hydronaphthylamin.

Pyretic: *Rabbit*, hypodermic, per kg., 25-50 mg. ($\frac{1}{2}$ to 1 c.c. of 5 per cent.) (Chap. 39, V).* (Details, Coleman, 1907; Ott and Scott, 1907; Jonescu, 1909.)

Bichromate, Potassium.

Fatal: *Dog*, stomach, 0.06 to 0.4 gm.

Bismuth Subcarbonate.

Antemetic: *Cat*, stomach, 1 gm. (Chap. 38, XVIII).*

Bromid, Sodium.

Antispasmodic: *Cat* or *Rabbit*, stomach, per kg., 2 gm. (10 c.c. of 20 per cent.) (Chap. 40, IV).

Bromural.

Effects: *Cats*, stomach, per kg.: Mean fatal, 0.45-0.5 gm.; Deep coma, 0.4 gm.; Light coma, 0.25-0.3 gm.; Sound natural sleep, 0.1-0.15 gm. (Sollmann and Hatcher, 1908).

Hypnotic: *Dogs*, stomach, per kg., 0.25 gm. (Gensler, 1915).

Brucin Hydrochlorid.

Effects: Hypodermic, per kg.:

	<i>Dog</i>	<i>Rabbit</i>	<i>Pigeon</i>	<i>Mouse</i>
Increased Reflexes.....	4.25 mg.	6.25 mg.	6.0 mg.	
Convulsive	4.5 mg.	7.5 mg.	26.5 mg.	40.3 mg.
Tetanic	7.5 mg.	8.6 mg.		
M. F. D.		18.5 mg.	42.2 mg.	108.2 mg.
<i>Dog</i> , rectal, per kg.:	4.25-16 mg., increased reflexes;	17-18 mg., tetanic.		

Cadmium Salts.

Fatal: *Dog* (large), hypodermic, 0.3 gm. *Rabbit*, stomach, 0.02-0.04 gm. *Frog*, lymph-sac, per gm., 1 mg.

Caffein (Free Alkaloid).

Ergograph: *Man*, 0.3 gm. (Chap. 33, V).*

Circulation and Respiration Stimulant, Diuretic, and Antidote: *Mammals*, vein and hypodermic, per kg., 10-20 mg. (1-2 c.c. of 1 per cent.) (Chap. 39, XVI, XVII; Chap. 41, I, 5; Chap. 41, VII, 2; Chap. 43, V, 9; Chap. 46, IV, 3).* (Details, Sollmann and Pilcher, 1911, Jour. Pharmacol., 3, 19.)

Toxic Dose: *Mammals*, vein or hypodermic, per kg., 40-100 mg. (4-10 c.c. of 1 per cent.) (Chap. 39, XVI).*

Convulsions and Rigor: *Frog*, lymph-sac, 10 mg. (1 c.c. of 1 per cent.) (Chap. 32, I, 11).*

Reflexes: *Frog*, lymph-sac, 5 mg. ($\frac{1}{2}$ c.c. of 1 per cent.) (Chap. 32, III, 5).*

Aorta: *Frog*, $\frac{1}{2}$ -1 c.c. of 1 per cent. (Chap. 32, I, 12).*

Caffein (*Continued*).

Fatal Dose, per kg.:	Vein	Hypodermic	Stomach
<i>Dog</i>		0.11 gm. (sometimes)	0.14 gm. (sometimes)
<i>Cat</i>		0.15 gm.	
<i>Rabbit</i>	0.16 gm.	0.28 gm.	0.36 gm.
<i>Guinea-pig</i>		0.28 gm.	

(Salant and Rieger, 1910.)

Calabarin.Fatal Convulsions: *Rabbit*, hypodermic, per kg., 20 mg.**Calcium Chlorid.**M. F. D. *Dog*, vein, per kg., 0.444 gm. (4 c.c. of m/8) (Joseph and Meltzer, 1909).Antagonism to Mg.: *Rabbit*, vein, 0.180–0.240 gm. (6–8 c.c. of 3 per cent.) (Chap. 39, XV).*Coagulation Time: *Rabbit*, hypodermic, 0.1 gm. (1 c.c. of 10 per cent.): effect in one to three hours (Coleman, 1907).**Calcium Lactate.**Against Inflammation and Effusions: *Dog* or *Cat*, hypodermic, per kg., 20 gm. (2 c.c. of 1 per cent.) (Chapter 37, XII and XIII).***Calomel.**Purgative: *Dog*, stomach, per kg., 0.16 gm. (Valeri, 1909).Systemic Effects: *Dog*, stomach, per kg., 0.21 gm. (Valeri, 1909).Diuresis and Enteritis: *Rabbit*, hypodermic, per kg., 5–10 mg. (1–2 c.c. of 0.5 per cent. in sod. thiosulphate).**Camphor.**Stimulant Dose: *Mammals*, vein, per kg., 5 to 30 mg. ($\frac{1}{2}$ to 3 c.c. of 1 per cent. in 40 per cent. alcohol) (Chap. 41, VII, 3; 45, V, 4).* (Details, Gottlieb, 1905.)*Rabbit*, hypodermic, per kg., 0.1 gm. ($\frac{1}{2}$ c.c. of 20 per cent.) (Chap. 41, I, 4).Convulsant, Ordinary Dose: *Cat* or *Rabbit*, stomach, per kg., 2 gm. (10 c.c. of 20 per cent. in oil) (Chap. 40, III).* *Frog*, 0.1 gm. (1 c.c. of 10 per cent.) (Chap. 32, I, 9) (also Curare Action, Chap. 32, IV, 10).

Minimum Convulsant Dose, per kg.:

	Stomach	Hypodermic	Intramuscular.
Dissolved in oil: <i>Dog</i>	0.5 gm.	0.75 gm.	0.5 gm.
Dissolved in alcohol: <i>Dog</i>	0.5 gm.	1.5 gm.	0.75 gm.
Dissolved in oil: <i>Cat</i>	0.25 gm.	0.5 gm.	0.5 gm.
Dissolved in alcohol: <i>Cat</i>	0.5 gm.	0.5 gm.	0.7 gm.

(Hatcher and Eggleston, 1914, Jour. Amer. Med. Assoc., 63, 469.)

M. F. D.: *Frog*, lymph-sac, per gm., 3.2 mg. (as 10 per cent. in oil) (Grove, 1910).*Guinea-pig*, mouth, per 100 gm., 0.15–0.18 gm. (Cairis, 1914, Jour. Pharm. Chem., 10, 224; also other methods of administration.)**Cane-sugar.**Comb: *Rooster*, pectoral muscles, per kg., 10 gm.: blueing in one-quarter to one-half hour.**Cannabis.**Narcosis: *Dog*, stomach, per kg., 0.05 gm. of Extract (Chap. 39, XIV).*Test Dose: *Dog*, stomach, per kg., 0.004 gm. of Extract (Chap. 39, XIV), *Dog*, stomach, per kg., 0.03 gm. of Fldext.Ataxia: *Cat*, hypodermic, $\frac{1}{2}$ c.c. of Tinct. with $\frac{1}{2}$ c.c. water (Dixon).**Cantharidin.**Vesicant: *Man*, 0.15 mg. local.Nephritis: *Mammals*, hypodermic or vein, per kg., 1 to 10 mg. (in acetic ether), severe (Chap. 39, XXI).* Glomerular only, 0.1 to 1 mg. per kg.**Caramel.**Antidote: *Cat*, stomach, 10 gm. (Chap. 40, VII).***Cascara.**Urine: *Man*, 1 c.c. of Fldext. (Chapter 15, XII).***Cephaëlin.**M. F. D.: *Mammals*, hypodermic, per kg., 30 mg.Emetic: *Dog*, stomach, per kg., 1 mg.**Cerium Oxalate.**Innocuous: *Rabbit*, stomach, per kg., 0.7 gm. (Bachem, 1907).**Cevadin.** See also *Veratrin*.Vagus Stimulation: *Mammals*, vein, per kg., 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) (Chap. 44, I, 3).*

Cevadin (*Continued*).

Convulsive: *Rabbits*, hypodermic, per kg., 3 mg.*

M. F. D.: *Rabbit* and *Guinea-pig*, hypodermic, per kg., 3-6 mg.*

Fatal: *Rabbit*, stomach, per kg., 10 mg.*

Charcoal.

Antidote: *Cat*, stomach, 10 gm. (Chap. 40, VII).*

Chloral.

Ordinary Dose (Anesthetic, narcotic, temperature, antidote, etc.): *Cat*, stomach, per kg., 0.25 gm. (2.5 c.c. of 10 per cent.) (Chap. 39, I, XVII; 40, X).* *Dog*, stomach, per kg., 0.25 to 0.3 gm.; vein, per kg., 0.1 to 0.15 gm. (Chap. 41, TN¹). *Rabbit*, stomach, per kg., 0.6 gm.*; rectum, per kg., 0.3 gm. (Chap. 41, IV, 3).*

Successive Effects: *Cat*, stomach, per kg., 0.09 to 0.15 gm.: natural sleep; 0.18 to 0.25 gm.: light coma; 0.30 gm. up: deep coma; 0.42 to 0.45 gm.: mean fatal dose. (Sollmann and Hatcher, 1908).*

Circulatory Depression: *Mammals*, vein, per kg., 0.5 gm. (5 c.c. of 10 per cent.) (Chap. 43, IV, 8).*

Respiratory Depression: *Rabbit*, stomach, per kg., 0.5 gm. (20 c.c. of 2.5 per cent.) (Chapter 41, IV, 3).*

Narcotic: *Frog*, lymph-sac, 0.02 gm. (1 c.c. of 2 per cent.) (Chap. 32, II, 4).*

Cardiac Depression: *Frog*, lymph-sac, 0.04 gm. (0.4 c.c. of 10 per cent.) (Chap. 36, V, 1).*

Fatal: *Frog*, lymph-sac, 0.1 gm. (1 c.c. of 10 per cent.).

Chloralose.

Anesthetic: *Dog*, stomach, per kg., 0.1 gm. (Pawlow).¹

Chlorate, Potassium.

Fatal: *Rabbit*, stomach, per kg., 4 gm.: methemoglobin cyanosis, respiratory paralysis, convulsions, death in four hours.

Chloretone.

Anesthetic (after morphin): *Dog*, stomach, per kg., 0.2 to 0.25 gm. (in alcohol) (Chap. 41, TN).* *Cat*, stomach, per kg., 0.3 gm. (in alcohol) (Chap. 41, TN).* *Rabbit*, stomach, per kg., 0.15 to 0.2 gm. (in alcohol) (Chap. 41, TN).*

Circulatory Depression: *Dog*, vein, per kg., 0.5 c.c. of saturated watery solution.*

Chlorid, Sodium.

Saline Infusion: *Mammals*, vein, per kg., 25 to 100 c.c. of 0.9 per cent.*

Diuretic: *Mammals*, vein, per kg., 2.5 c.c. of 10 per cent. (Chap. 46, III, 2).*

Fatal: *Mammals*, vein, per kg., 10 to 30 c.c. of 10 to 33 per cent.; death in four to five minutes.* *Dog*, vein, per kg., 64 c.c. of m/8. (Joseph and Meltzer, 1909.)

Chloroform.

Anesthetic: *Mammals*, vein, per kg., 1 c.c. of 0.5 per cent. (Chap. 42, III, 14).*

Narcotic: *Frogs*, lymph-sac, 0.2 gm. (1 c.c. of 20 per cent. in oil) (Chap. 32, II, 6).*

Fatal: *Frogs*, lymph-sac, 0.45 gm.

Kidney Lesions (Fiske and Karsner, 1914).

Cholin.

Ordinary Dose: *Mammals*, vein, per kg., 1 to 2 mg. (1 to 2 c.c. of 1 : 1000) (Chap. 43, II, 8).* (Details, Abderhalden and Mueller, 1911; Busquet and Pachon, 1912).

Chromate, Potassium. See *Bichromate*.

Fatal: *Rabbit*, hypodermic, 0.2-0.4 gm.; stomach, 2 gm.

Nephritis: *Dog*, hypodermic, per kg., 2.5-50 mg.; vein, per kg., 5 mg. (MacNider, 1912). *Rabbit*, hypodermic, per kg., 30 mg. (Chap. 39, XXI).* *Guinea-pig*, hypodermic, per kg., 50 mg. (nearly fatal, Ophüls, 1911).

Urate Deposits: *Pigeons*, 10 mg.; *Hens*, 10 to 20 mg. (hypodermic, repeated several days).

Citrate, Sodium (Crystals).

Urine: *Man*, stomach, 10 gm., alkaline reaction (Chap. 15, VI).*

M. F. D.: *Dog*, vein, per kg., 0.37 gm. *Frog*, lymph-sac, per gm., 4 to 5 mg.

Cobalt, Nitrate.

M. F. D.: *Mammals*, hypodermic, per kg., 50 to 75 mg. *Pigeon*, hypodermic, per kg., 5 to 10 mg. *Frog*, hypodermic, per kg., 1.8 to 4 mg.

Cocain Hydrochlorid.

(Doses for mammals, Grode, 1912, Arch. exp. Path. Pharm., 57, 172.)

Temperature: *Mammals*, hypodermic, per kg., 25 mg. (0.5 c.c. of 5 per cent.) (Chap. 39, IV).*

M. F. D.: *Rabbit*, per kg., hypodermic, 0.1 to 0.12 gm.; vein, 0.01 to 0.022 gm.; peritoneum, 0.015 to 0.02 gm. *Guinea-pig*, hypodermic, per kg., 0.05 to 0.06 gm. *Frog*, lymph-sac, 3 mg.

¹ TN= Technical Notes.

Cocain Hydrochlorid (*Continued*).

Intravenous Anesthesia: *Rabbit*, ear vein, per kg., 10 mg. (1 c.c. of 1 per cent.) (Ritter, 1909; Chap. 32, V, 11).

Effects: *Dog*, hypodermic, 2.5 mg. \times kg., raises temperature by 0.2° to 0.5° C. for two hours; 10 mg. \times kg. by 1° to 2° for three to four hours; 20 mg. \times kg., 2° to 4° for six to seven hours; 15 to 20 mg. \times kg. emesis, mydriasis, convulsions, and paralysis, with recovery; 25 mg. \times kg., sometimes fatal; 80 mg. \times kg., sometimes recovery. *Rabbit*, hypodermic, 20 mg. \times kg., ordinary dose for hyperpyrexia (0.25° to 0.8° in one to three hours) (*); 30 mg. \times kg., slight trembling; 50 mg. \times kg., considerable rise of temperature; 60 mg. \times kg., convulsions, paralysis, recovery; 100 mg. \times kg., sometimes fatal; 130 mg. \times kg., sometimes recovery; 540 mg. \times kg., surely fatal.

Cocculus.

Fatal: *Dog*, hypodermic, per kg., 0.4 gm.

Convulsant: *Frog*, lymph-sac, per gm., 2 mg.

Codein.

Respiration: *Mammals*, hypodermic, per kg., 5 to 10 mg.

M. F. D.: *Cats*, hypodermic, per kg., 60-90 mg. (Mueller, 1908). *Rabbit*, hypodermic, per kg., 60 mg.

Toxic: *Frog*, lymph-sac, 10 mg. (1 c.c. of 1 per cent.) (Chap. 32, II, 6).*

Colchicin.

Leukocytosis: *Rabbit*, hypodermic, per kg., 5 mg. (1 c.c. of 0.5 per cent.); maximum in twelve hours (Coleman, 1907).

M. F. D.: *White Mice*, per kg., 1-25 mg. (Fuehner, 1910).

Colchicum (Seed).

(Colchicum root requires about twice these doses).

Fatal Gastro-enteritis: *Dog* or *Cat*, stomach, per kg., 0.5 c.c. of Fldext. (Chap. 39, XVIII, 1).*

Colocynth.

Purgative: *Cat*, stomach, 10 c.c. of 10 per cent. decoction: liquid stools in one to four hours.

Coniin.

Fatal: *Cat*, hypodermic, 0.4 gm., fatal in one hour; 0.05 gm., fatal in nine hours.

Rabbit, hypodermic, per kg., 90 mg. *Pigeon*, hypodermic, per kg., 40 mg.

Mouse, hypodermic, per kg., 75 mg.

Paralytic: about three-quarters of the fatal dose.

Curare Action: *Frog*, lymph-sac, 10 mg. (1 c.c. of 1 per cent.) (Chap. 32, IV, 10).

Conium.

Ineffective: *Dog*, hypodermic, per kg., 0.5 gm.*

M. F. D.: *Guinea-pig*, hypodermic, per kg., 0.5 gm.* *White Rat*, hypodermic, per kg., 40 gm.* *Frog*, lymph-sac, per gm., 0.06 gm.*

Convallaria.

M. F. D.: *Guinea-pig*, hypodermic, per kg., 0.08 gm. *Rat*, hypodermic, per kg., 32 gm. *Frog*, hypodermic, per gm., 0.18 mg.

Copaiba.

Urine: *Man*, 1 gm.*

Copper Sulphate.

Emetic: *Cat*, stomach, 25 c.c. of 1 per cent. (Chap. 38, XVI).* *Dog*, stomach, 50 c.c. of 1 per cent.*

Coriamyrtin.

Convulsive: *Dog*, hypodermic, per kg., 0.15 mg. *Rabbit*, hypodermic, per kg., 0.75 mg.

Fatal: *Cat*, hypodermic, per kg., 0.25 mg. *Guinea-pig*, hypodermic, per kg., 2.5 mg.,

Frog, hypodermic, per kg., 0.1 mg.

Cotarnin, Hydrochlorid or Phthalate.

Circulatory: *Mammals*, vein, per kg., 5 mg. ($\frac{1}{2}$ c.c. of 1 per cent.) (Chap. 43, II, 9).*

Curare.

Paralytic: *Mammals*, vein, per kg., 3 mg. ($\frac{2}{3}$ c.c. of $\frac{1}{2}$ per cent.), repeated every ten minutes as needed (Chap. 32, IV, 6; 42, VI, 3).* *Frog*, lymph-sac, 2.5-5 mg. ($\frac{1}{2}$ to 1 c.c. of $\frac{1}{2}$ per cent.) (Chap. 32, IV, 1)*; immersion of muscle: $\frac{1}{10}$ per cent. (Chap. 32, IV, 4).*

Curarin.

Paralytic: *Mammals*, vein, per kg., 0.5 to 3 mg. *Frog*, lymph-sac, per gm., 0.00025 to 0.001 mg.

Cyanid, Potassium.

Toxic: *Mammals*, vein, per kg., 5 mg. ($\frac{1}{2}$ c.c. of 1 per cent.) (Chap. 43, I, 15).*

M. F. D.: *Rabbit*, hypodermic, per kg., 1.0 mg. (no effect below 1 mg.). *Mouse*, hypodermic, per kg., 4.4 mg. *Pigeon*, hypodermic, per kg., 1.5 to 2.4 mg.

Cystisin.

Fatal: *Cat*, hypodermic, 30 to 40 mg.

Delphinin (Heyl).

Vagus Paralysis: *Rabbit*, hypodermic, 75 mg. (1.5 c.c. of 5 per cent.).

Fatal: *Dog* or *Cat*, 0.03 to 0.1 gm.

Digitalis.

Circulatory, Ordinary: *Mammals*, vein, per kg., 50 mg. (1 c.c. of 5 per cent.) (Chap. 45, III, 4).*

Circulatory, Toxic: *Mammals*, vein, per kg., 100 mg. (2 c.c. of 5 per cent.) (Chap. 45, III, 4).* *Frog*, lymph-sac, 25 mg. (0.5 c.c. of 5 per cent.) (Chap. 36, IV).*

M. F. D.: *Frog*, lymph-sac, per gm., 0.6 mg. (Chap. 36, III).* (Hatcher, 1912): *Cat*, vein, per kg., 0.1 gm. *Dog*, vein, per kg., 0.125 gm. *Rabbit*, vein, per kg., 0.2 to 0.25 gm.

Digitaloid Drugs.

M. F. D.: *Cats*, *Frogs*, and *Guinea-pigs* (Chap. 36, III). *Cats*, vein, (Hatcher, Arch. Int. Med., Sept., 1912).

Digitoxin.

M. F. D. (Hatcher, 1912): *Cat*, vein, per kg., 0.3 mg. *Dog*, vein, per kg., 0.5 mg. *Rabbit*, vein, per kg., 0.75 to 1 mg.

Dionin.

Respiration: *Rabbit*, hypodermic, per kg., 6 mg.

Fatal: *Rabbit*, hypodermic, per kg., 100 mg.

Local, *Eye*: 10 per cent.

Diphtheria Toxin.

(Meyer, Arch. exp. Path., 60.)

Diuretin. See *Theobromin*.**Emetin.**

Just Emetic: *Dog*, hypodermic, per kg., 1½ mg.; vein, 5 mg. per kg.

Fatal: *Mammals*, hypodermic, per kg., 0.1 gm.; vein, per kg., 0.02 gm. *Frog*, lymph-sac, 10-20 mg.

Paralysis: *Frog*, lymph-sac, 5 mg.

Local: *Dog's conjunctiva*, 1 : 500; irritant.

Epinephrin (Adrenalin).

Blood-pressure: Ordinary Dose: *Mammals*, vein, per kg., 0.02 to 0.05 mg. ($\frac{1}{5}$ to $\frac{1}{2}$ c.c. of 1 : 1000) (Chap. 43, I, 9).*

Minimal Rise: *Atropinized dog*, vein, per kg., 0.0001 mg (Toujan, 1905). *Rabbit*, vein, per kg., 0.0003 mg. (Cameron, 1905).

Maximal Rise: *Cat*, vein, per kg., 0.03 mg. (Elliott, 1905). *Rabbit*, vein, per kg., 0.047 mg. (Pruszyński, 1905).

Progressive Rise: *Atropinized dog*, vein, per kg. (Hunt, 1901, and others):

0.000085 mg.	=	5 mm. rise.
0.00025 mg.	=	7 mm. "
0.0005 mg.	=	15 mm. "
0.0007 mg.	=	20 mm. "
0.0017 mg.	=	25 mm. "
0.004 mg.	=	45 mm. "
0.0055 mg.	=	65 mm. "
0.03 mg.	=	150 mm. "

Bronchial Relaxation: *Rabbit*, vein, 0.1 mg. (1 c.c. of 1 : 10,000) (Chap. 37, VII).*

Glycosuria: *Rabbit*, hypodermic, 1 to 2 mg. (1 to 2 c.c. of 1 : 1000) (Chap. 39, X).*

Myocarditis: *Rabbit*, vein, 0.2 mg. (0.2 c.c. of 1 : 1000), with 12 mg. of Spartein Sulphate or of Caffein.

Pupil: *Frog*, lymph-sac, 0.1 c.c. of 1 : 1000 (Meltzer and Auer, 1904).

M. F. D.: *Dog*, vein, per kg., 0.1 to 0.25 mg. (Lesage, 1904); hypodermic, per kg., 5 to 6 mg. *Cat*, vein, per kg., 0.5 to 0.8 mg. (Lesage). *Rabbit*, vein, per kg., 0.2 to 0.6 mg.; hypodermic, per kg., 2.5 to 10 mg. (Paton, 1905; Baylac). *Guinea-pig*, vein, per kg., 0.1 to 0.2 mg.; hypodermic, per kg., 4 to 10 mg. (Baylac).

Ergot.

Circulation: *Mammals*, vein, per kg., 0.025 to 0.25 gm. ($\frac{1}{10}$ to 1 c.c. of 25 per cent.) (Chap. 43, II, 5).*

Rooster (Comb): hypodermic, 5 gm. (Chap. 35, II)*; stomach, 15 to 30 gm. (Crawford, 1908).

Uterus: *Cat* or *Rabbit*, vein, 0.2 to 0.3 gm. (Edmunds and Roth, 1908).

M. F. D.: *Guinea-pig*, hypodermic, per kg., 8 gm. *Frog*, lymph-sac, per gm., 50 mg.

Ergotoxin.

Circulation: *Mammals*, vein, per kg., 0.25 to 0.5 mg. ($\frac{1}{4}$ to $\frac{1}{2}$ c.c. of 1 : 1000) (Chap. 43, I, 15).

Ether.

Anesthetic: *Mammals*, vein, per kg., $\frac{1}{4}$ to $\frac{1}{2}$ c.c. of saturated solution (Derouaux, 1909) (Chap. 42, III, 14).*

Stimulant: *Mammals*, hypodermic, 5 c.c.*

Eucaïn, Beta-, Hydrochlorid.

M. F. D.: *Rabbit*, hypodermic, per kg., 0.4 to 0.5 gm. (Vinci). *Guinea-pig*, hypodermic, per kg., 0.3 to 0.35 gm. (Vinci).

Ferrocyanid, Sodium.

Non-toxic: *Dog*, vein, per kg., 35 c.c. of 7.5 per cent. crystals.*

Filmaron.

Anthelmintic: *Dogs*, 0.2 to 1 gm., in capsules, followed by purgative (Gmeiner, 1907; Merck's Rep., 21, 108).

Fluorid, Sodium.

M. F. D.: *Dog*, vein, per kg., 0.05 to 0.1 gm.; hypodermic, per kg., 0.15 gm. *Rabbit*, vein, per kg., 0.14 gm.; hypodermic, per kg., 0.15 gm.; stomach, per kg., 0.5 gm. *Frog*, lymph-sac, 40 mg.

To kill epithelium, 0.03 to 0.3 per cent.; preservative, 0.2 per cent.; muscle twitchings, 0.5 per cent.

Formaldehyd.

M. F. D.: *Dog*, vein, per kg., 0.07 gm.; hypodermic, per kg., 0.35 gm. (twenty-four hours). *Rabbit*, vein, per kg., 0.09 gm.; hypodermic, per kg., 0.22 to 0.5 gm. (several days). *Guinea-pig*, hypodermic, per kg., 0.8 gm. *Frog*, lymph-sac, 0.8 mg.

Formate, Sodium.

M. F. D.: *Dog*, stomach, per kg., 4 gm.; vein, per kg., 3 gm. *Rabbit*, somewhat larger (Fleig, 1907).

Fuchsin, Acid.

Convulsions: *Frog*, lymph-sac, per gm., 0.03 c.c. of 5 per cent. (Chap. 32, I, 7).*

Gelseminin Hydrochlorid.

Toxic: *Frog*, lymph-sac, 20 mg.

Gelsemium.

M. F. D.: *Guinea-pig*, hypodermic, per kg., 1.75 to 6 gm. *White Rat*, hypodermic, per kg., 2.2 gm. *Frog*, lymph-sac, per gm., 6.5 to 15 mg.

Glucose.

Diuretic: *Mammals*, vein, per kg., 25 c.c. of 6 per cent. (Chap. 46, IV, 1).*

Glycerin.

Muscular: *Frog*, lymph-sac, 0.5 to 1 c.c.

Gold (Calculated as Metal).

M. F. D.: *Dog*, hypodermic, per kg., 0.4 gm.; ditto, *Cat*, 0.45 gm.; ditto, *Rabbit*, 0.36 gm.; ditto, *Frog*, 0.30 gm.

Grehant Anesthetic.

Anesthetic: *Dogs*, per kg.: Morphine, hypodermic, $\frac{1}{4}$ of 4 per cent.; Grehant Mixture, stomach, 6 to 10 c.c. (Chap. 41, TN).* (Mixture contains 5 per cent. of chloroform in 50 per cent. alcohol.)

Guanidin.

(Fuehner, 1907, Arch. exp. Path. Pharm., 58, 1.)

Hedonal.

Rabbit, stomach, per kg.: sleep, 0.1 to 0.2 gm.; anesthetic, 0.25 gm.; toxic, 0.35 gm. (Cataldi, Wien. med. Presse, 1906, No. 50.)

Helleborein.

M. F. D.: *Frog*, lymph-sac, per gm., 0.004 mg; ditto, *Toad*, 0.185 to 0.244 mg.

Helleborus Niger.

M. F. D.: *Guinea-pig*, hypodermic, per kg., 0.2 gm.; ditto, *White Rat*, 20 gm.; ditto, *Frog*, 0.3 gm.*

Heroin.

Respiration: *Rabbit*, hypodermic, per kg., 0.5 mg.*

Hexamethylenamin.

Excretion: *Man*, stomach, 0.5 gm. (Chap. 15, V).* *Dog*, stomach, per kg., 0.5 gm. (Chap. 38, IX).*

M. F. D.: *Guinea-pig*, hypodermic, per kg., over 10 gm. (Frothingham, 1909).

Hirudin.

Coagulation: *Mammals*, vein, per kg., 20 to 50 mg. Direct addition to blood, 1 : 6000.

Histamin.

Bronchi and Circulation: *Mammals*, vein, per kg., 0.01 to 0.1 mg. ($\frac{1}{10}$ to 1 c.c. of 1 : 10,000) (Chap. 37, VII; 43, II, 7).*

Fatal: *Rabbit*, vein, per kg., 0.5 mg. (Oehme, 1913).

Hordenin.

M. F. D.: *Dog* or *Rabbit*, vein, per kg., 0.25 gm. (Martinet, 1910).

Hydrastin Hydrochlorid.

Circulation: *Mammals*, vein, per kg., 5 mg. (5 c.c. of 1 : 1000) (Chap. 43, I, 15).*

(Williams, 1908; Marfori, 1890.)

Convulsions: *Frog*, lymph-sac, 1 to 2 c.c. of 1 : 1000 (Chap. 32, I, 9).*

Hydrastinin Hydrochlorid.

Circulation: *Mammals*, vein, per kg., 1 to 5 mg. ($\frac{1}{10}$ to $\frac{1}{2}$ c.c. of 1 per cent.) (Chap. 43, II, 11).* (Marfori, 1890; Williams, 1907.)

Paralytic: *Frog*, lymph-sac, 5 mg.; fatal, 15 mg. (Marfori, 1890).

Hydrastis.

Circulation: *Mammals*, vein, per kg., 20 mg. (1 c.c. of 2 per cent.) (Chap. 43, II, 10).*

(Details, Williams, 1907.)

Ineffective: *Dog*, hypodermic, per kg., 0.2 gm.; ditto, stomach, 0.5 gm.

Convulsive: *Frog*, lymph-sac, 1 c.c. of 5 per cent.

Hydrazin Sulphate.

Fatal Convulsions: *Rabbit*, hypodermic, per kg., 0.315 gm.

Kidney Lesions: *Cat*, hypodermic, per kg., 0.1 gm., forty-eight hours before use (Fiske and Karsner, 1914).

Hydrocyanic Acid ($\frac{1}{2}$ of Cyanid of Potassium).

M. F. D.: *Cat*, per kg., 0.8–1 mg. (Lehmann).

Surely Fatal: *Cat*, stomach, per kg., 2 mg. (2 c.c. of 1 : 1000) (Chap. 40, VI, 2).*

Cat or *Rabbit*, mouth, 1 c.c. of 2 per cent.; ditto, *Dog*, 5 c.c. of 2 per cent. (Chap. 38, II, 2).*

Hyoscin. See *Scopolamin*.**Hyoscyamin.**

Mydriasis: *Cat*, hypodermic, per kg., 0.02 mg.

Minimum Vagus Paralysis: *Cat*, hypodermic, per kg., 0.025 mg.

Toxic: *White Mice*, hypodermic, per 12–15 gm., 10 mg.

Fatal: *White Mice*, hypodermic, per 12–15 gm., 20 mg.

Motor depression: *Frog*, lymph-sac, per 20 gm., 2 mg.

Fatal: *Frog*, lymph-sac, per 20 gm., 10 mg.

Hyoscyamus.

M. F. D.: *Guinea-pig*, hypodermic, per kg., 10 gm.* *Frog*, lymph-sac, per gm., 10 mg.*

Hyposulphite. See *Thiosulphate*.**Iodid, Potassium.**

Excretion: *Man*, mouth, 0.3 gm. (Chap. 15, I).*

Iodid, Sodium.

Not toxic: *Dog*, vein, per kg., 35 c.c. of 2.2 per cent.*

Pleural effusion: *Dog*, vein, per kg., 1 c.c. of 10 per cent. (Chap. 37, XIII).*

Depression: *Rabbit*, stomach, 50 c.c. of 1 per cent.*

Iodin.

Fever: *Rabbit*, hypodermic, 2 c.c. of tincture.

Fatal: *Rabbit*, hypodermic, 0.075 gm.

Iodoform.

Hypnotic: *Rabbit*, hypodermic, per kg., 2 gm. in oil.

Fatal: *Rabbit*, stomach, 1 to 2 gm.

Ipecac.

Just emetic: *Dog*, stomach, per kg., 0.2 to 0.3 gm. (Chap. 38, XVI).

Systemic, toxic: *Dog*, hypodermic, per kg., 1 gm.*

Iron (Calculated as Metal).

M. F. D.: *Dog*, hypodermic, 2 to 50 mg.; ditto, *Rabbit*, 25 mg. *Frog*, lymph-sac, per kg., 5 to 10 mg.

Isopral.

Effects: *Cat*, stomach, per kg. (Sollmann and Hatcher, 1908): Sound natural sleep, to 0.1 gm.; light coma, 0.11 to 0.18 gm.; deep coma, above 0.18 gm.; mean M. F. D., 0.25 to 0.30 gm.

Juniper Oil.

Diuretic: *Dog*, vein, per kg., 25 c.c. of 0.4 per cent. suspension.

Laudanin.

Convulsions: *Rabbit*, hypodermic, per kg., 20 mg.

M. F. D.: *Rabbit* or *Dog*, hypodermic, per kg., 30 mg.

Laudanosin.

M. F. D.: *Rabbit*, hypodermic, per kg., 68 mg.

Lead Acetate.

Intestinal Spasm: *Mammals*, vein, per kg., 5 to 8 mg. (Hirschfelder, 1915).

Leeches.

Coagulation: *Mammals*, vein, per kg., 3 heads in 6 c.c. of normal saline.

Lobelia.

Circulatory: *Dog*, hypodermic, per kg., 0.33 gm.*

M. F. D.: *Guinea-pig*, hypodermic, per kg., 10 gm.* *Frog*, lymph-sac, per gm., 55 mg.*

Lobelin Sulphate.

Respiratory Stimulation: *Rabbit*, hypodermic, per kg., 2 mg.

Phrenic Paralysis: *Rabbit*, vein, per kg., 8-12 mg.

Reflexes: *Frog*, lymph-sac, 3 mg.

Curare Action: *Frog*, lymph-sac, 10 mg. (Chap. 32, IV, 10); immersion of muscle, 0.2 per cent.

M. F. D.: *Pigeon*, hypodermic, per kg., 54 mg.

Magnesium Chlorid.

M. F. D.: *Dog*, vein, per kg., 0.223 gm. (2.35 c.c. of M/8). (Joseph and Meltzer, 1909.)

Magnesium Sulphate (Crystals).

Anesthetic: *All animals*, hypodermic, per kg., 1.5 to 1.75 gm. (6 to 7 c.c. of 25 per cent.). (*Rabbit*, Chap. 39, XV; *Frog*, 0.8 c.c. of 25 per cent. per gm., Chap. 32, II, 6.)* (*Curare Action*, immersion of muscle, 5 per cent., Chap. 32, IV, 4.)*

Fatal: *All animals*, hypodermic, per kg., 2 gm.

Manganese (Calculated as Metal).

M. F. D.: *Dogs*, hypodermic, per kg., 10 to 13 mg.; *Cat*, ditto, 6 to 7 mg.; *Rabbit*, ditto, 5 to 6 mg.

Mercuric Chlorid.

Gastro-enteritis: *Cat* or *Rabbit*, stomach, per kg., 5 mg. (5 c.c. 1 : 1000) (Chap. 39, XVIII, 2).*

Nephritis: *Dog* or *Rabbit*, hypodermic, 5 to 10 mg. (5 to 10 c.c. of 1 : 1000) (Chap. 39, XXI; MacNider, 1912).

Methylene-blue.

Excretion: *Man*, mouth, 0.15 gm. (Chap. 15, VII, A).*

Fatal: *Dog*, vein, per kg., 0.125 gm. (25 c.c. of 0.5 per cent.); stomach, per kg., over 1 gm. (Tanfiljeff, 1907).

Morphin Hydrochlorid or Sulphate.

Respiration: Sedative: *Rabbit*, hypodermic, per kg., 0.5 mg. ($\frac{1}{2}$ c.c. of 1 : 1000) (Chap. 41, I, 1).* *Cat*, hypodermic, per kg., 2.5 mg.

Maximal: *Rabbit*, hypodermic, per kg., 2.5 to 10 mg.

Toxic: *Rabbit*, hypodermic, per kg., 40 mg. (1 c.c. of 4 per cent.) (Chap. 41, I, 3).*

Temperature: *Rabbit*, hypodermic, per kg., 100 mg. (2.5 c.c. of 4 per cent.) (Chap. 39, II).* *Dog*, hypodermic, per kg., 10 to 150 mg.

Gastric Spasm: *Dog*, hypodermic, per kg., 6 to 7 mg.; *Cat*, ditto, 8 mg.

Antemetic: *Dog*, hypodermic, per kg., 10 mg. ($\frac{1}{4}$ c.c. of 4 per cent.) (Chap. 38, XVII).*

Excitement: *Cat*, hypodermic, per kg., 40 mg.

Constipation: *Cat*, hypodermic, per kg., 40 mg. (milk diarrhea). *Rabbit*, hypodermic, per kg., 20 mg. (salt crystal to intestine; reappears with 60 mg.).

Glycosuria: *Rabbit*, hypodermic, per kg., 50 to 100 mg. (Chap. 39, VIII).*

Narcotic and Preliminary Anesthetic: *Dog*, hypodermic, per kg., 10 to 20 mg. ($\frac{1}{4}$ to $\frac{1}{2}$ c.c. of 4 per cent.) (Chap. 39, XIII; 41, TN).* *Cat*, hypodermic, per kg., 20 to 60 mg. ($\frac{1}{2}$ to $1\frac{1}{2}$ c.c. of 4 per cent.) (Chap. 39, XIII; 41, TN).* *Rabbit*, hypodermic, per kg., 5 to 20 mg. ($\frac{1}{8}$ to $\frac{1}{2}$ c.c. of 4 per cent.) (Chap. 41, TN).*

Mouse Test: Ordinary: *White Mouse*, hypodermic, per 15 to 20 gm., 0.05 mg. (0.5 c.c. of 1 : 1000) (Chap. 39, XIII, 4).*

Minimal: *White Mouse*, hypodermic, per 15 to 20 gm., 0.01 mg.

Reflexes: *Frog*, 10 mg. ($\frac{1}{4}$ c.c. of 4 per cent.) (Chap. 32, III, 4).*

Narcotic, Tetanic: *Frog*, 50 mg. ($1\frac{1}{2}$ c.c. of 4 per cent.) (Chap. 32, II, 1).*

M. F. D.: *Dog*, vein or hypodermic, per kg., 0.4 gm. (Lenthaz). *Cat*, hypodermic, per kg., 0.04 to 0.08 gm. (G. H. Mueller, 1908). *Rabbit*, hypodermic, per kg., 0.2 to 0.32 gm. (Stockman, 1891; Joffroy and Lervaux): stomach, per kg., 0.7 to 1 gm. *Guinea-pig*, hypodermic, per kg., 0.7 gm. *White Rat*, hypodermic, per gm., 0.42 mg. (Hunt, 1907). *White Mouse*, hypodermic, per gm., 0.6 mg. (Hale, 1910).

Morphin-atropin Anesthesia.

Cat, hypodermic, per kg., Morphin, 20 mg. ($\frac{1}{2}$ c.c. of 4 per cent.), with Atropin, 1 mg. (1 c.c. of 1 : 1000) (Chap. 39, XIII, 7).*

Morphin-atropin-urethane Anesthesia.

Cat, rectal or stomach, per kg., 3 c.c. of the M. A. U. Mixture (1 c.c. = 10 mg. of Morphin, 0.2 mg. of Atropin, and 0.2 gm. of Urethane) (Chap. 41, TN).*

Morphin-scopolamin Anesthesia.

Dog or *Rabbit*, hypodermic, per kg., Morphin, 10 mg. ($\frac{1}{4}$ c.c. of 4 per cent.), with Scopolamin, 0.67 mg. ($\frac{3}{8}$ c.c. of 1 : 1000) (Chap. 42, II, 4; V).* (Details, Boytcheff, 1907.)

Cat, hypodermic, per kg., Morphin, 20 mg. ($\frac{1}{2}$ c.c. of 4 per cent.), with Scopolamin, 0.5 mg. ($\frac{1}{2}$ c.c. of 1 : 1000) (Chap. 39, XIII, 7).*

Muscarin Sulphate.

Cardiac: *Dog*, hypodermic, per kg., 2 mg.

Toxic: *Cat*, hypodermic, per kg., $\frac{1}{4}$ to $\frac{1}{2}$ mg.

Bronchial Constriction: *Cat*, hypodermic, per kg., $\frac{1}{2}$ mg.

Fatal: *Cat*, hypodermic, per kg., 1 to 2 mg. in two to twelve hours; 3 to 5 mg. in ten to fifteen minutes.

Vagus Stimulation: *Frog*, lymph-sac, 0.5 mg.

M. F. D.: *Frog*, lymph-sac, per 10 gm., 0.12 to 0.23 mg. *Toad*, lymph-sac, per 10 gm., 0.21 to 0.27 mg.

Mustard.

Emetic: *Dog*, stomach, teaspoon.

Naphthol, Beta-.

Anthelmintic: *Dog*, stomach, per kg., 0.06 gm.; *Cat*, ditto, 0.01 gm.

Fatal: *Cat*, stomach, per kg., 0.1 gm.

Narcein.

Respiratory sedative: *Cat*, stomach, 0.1 gm.

Narcotin.

Light Narcosis: *Dog*, hypodermic, per kg., 50 mg. *Mouse*, hypodermic, per 15 to 20 gm., 10 mg.; not fatal (Chap. 39, XIII, 5).* *Frog*, lymph-sac, 50 to 70 mg.

Fatal: *Cat*, 3 gm.

Neuronal.

Hypnotic: *Dog*, stomach, per kg., 0.1 gm. (Gensler, 1915).

Nickel. Same as *Cobalt*.**Nicotin.**

Stimulant, Circulation: *Mammals*, vein, per kg., 0.1 to 0.5 mg. ($\frac{1}{10}$ c.c. to $\frac{1}{2}$ c.c. of 1 : 1000) (Chap. 43, II, 12).*

Emetic: *Dog*, vein, per kg., 0.35 mg. (Eggleston, 1916) (Chap. 43, II, 12).*

Stimulant, Respiration: *Rabbit*, hypodermic, per kg., 0.5 mg. ($\frac{1}{2}$ c.c. of 1 : 1000) (Chap. 41, VI, 4).*

Stimulant, Peristalsis: *Rabbit*, hypodermic, per kg., 10 mg. (1 c.c. of 1 : 100) (Chap. 34, II).*

Dilation of Ear Vessels: *Rabbit*, hypodermic, per kg., 10 mg. (1 c.c. of 1 : 100) (Chap. 35, I).*

Sympathetic Paralysis: *Rabbits* or *Cats*, vein, per kg., 5 to 10 mg.; local, 1 per cent. (both uncertain in dogs) (Chap. 34, I, 7).*

Fatal Convulsions: *Dog*, mouth, 2 drops, undiluted; *Rabbit* or *Cat*, ditto, 1 drop (Chap. 38, II).*

Toxic: *Frog*, lymph-sac, 1 mg. (1 c.c. of 1 : 1000) (Chap. 32, IV, 8).*

Muscle: Immersion, $\frac{1}{10}$ per cent. (Chap. 32, IV, 4).*

M. F. D.: *Cat*, per kg., vein, 1.5 mg.; hypodermic, 5 mg.; stomach, 10 mg. *Rabbit*, per kg., vein, 10 mg.; hypodermic, 30 mg.; stomach, 30 mg. *Guinea-pig*, per kg., vein, 2.25 mg.; hypodermic, 10 mg.; stomach, over 200 mg. (Hatcher and Eggleston, 1914).

Nitrate, Sodium.

Non-toxic: *Dog*, vein, per kg., 75 c.c. of 1.25 per cent.*

Fatal: *Frog*, 0.03 gm.

Nitrite, Sodium.

Vascular: *Mammals*, vein, per kg., 5 to 30 mg. ($\frac{1}{20}$ to $\frac{1}{3}$ of 10 per cent.) (Chap. 43, III, 2).* (Details, Dossin, 1911.)

M. F. D.: *White Mouse*, hypodermic, per gm., 0.15 mg. (Hale, 1910).

Fatal, Methemoglobin: *Rabbit*, hypodermic, per kg., 10 mg. *Guinea-pig*, ditto, 150 mg.

Spinal Paralysis: *Frog*, lymph-sac, per gm., 0.55 mg.

Nitroglycerin.

Vascular: *Mammals*, vein, per kg., 0.5 mg. ($\frac{1}{2}$ c.c. of 1 per cent.) (Chap. 43, I, 4).
 Minimal: *Rabbit*, vein, per kg., 0.05 mg. (Edmunds and Roth, 1908).

Novocain.

M. F. D.: *Cat*, peritoneum, per kg., 0.45 gm. *Dog*, peritoneum, per kg., 0.4 gm.; vein, per kg., 0.2 gm.

Nuclein.

Leukocytosis: *Rabbit*, hypodermic, 0.5 c.c. of $\frac{1}{2}$ per cent. (in eight hours; Coleman, 1907).

Oleate, Sodium.

Hemolysis: *Dog*, vein, per kg., 10 c.c. of 1 per cent.

Optochin.

M. F. D.: *Frog*, lymph-sac, per gm., 0.30 mg. *White Mice*, ditto, 0.5 mg. (Smith and Fantus, 1916).

Ouabain (g-Strophanthin).

Circulation: *Mammals*, vein, per kg., 0.05 mg. ($\frac{1}{2}$ c.c. of 1 : 1000) (Chap. 44, III, 5).
 M. F. D.: *Cat*, vein, per kg., 0.1 mg. (Chap. 36, III, 5). *Dog*, vein, per kg., 0.125 to 0.175 mg.; *Rabbit*, 0.2 mg. (Hatcher, 1912). *Frog*, lymph-sac, per gm., 0.0005 mg. (Chap. 36, III, 1).
 Heart: *Frog*, lymph-sac, $\frac{1}{2}$ c.c. of 1 : 50,000 (Chap. 36, IV, 2).

Oxalate, Sodium.

Kidney Deposits: *Rabbits*, hypodermic, 0.25 gm., fatal in few hours.

M. F. D.: *Guinea-pig*, hypodermic, per kg., 0.4 gm. (Chap. 39, XXI). *Chicken*, ditto, 0.5 gm. *Turtle*, ditto, 0.26 gm. *Frog*, ditto, 0.5 gm.

Anticoagulant: *Blood*, 0.2 to 1 : 300.

Oxalic Acid.

Fatal: *Rabbit*, stomach, 2 to 4 gm. (fatal in one-quarter to one-half hour). *Guinea-pig*, hypodermic, 0.1 gm. *Frog*, lymph-sac, 40 to 80 mg.

Oxydimorphan.

M. F. D.: *Dog*, vein, per kg., 60 mg. (dissolve in 0.2 per cent. NaOH); hypodermic, not fatal in any dose.

Papaverin Hydrochlorid.

Respiration: *Cat*, hypodermic, per kg., 40 mg.

Narcotic: *Cat*, hypodermic, per kg., 100 mg. (Chap. 39, XIII, 6).

M. F. D.: *Cat*, hypodermic, per kg., 128 mg. (G. H. Mueller, 1908).

Paraldehyd.

Anesthesia: *Rabbit*, stomach, per kg., 1 gm. (Chap. 41, TN)* (Mansfeld, 1905).

Vasomotor Paralysis: *Rabbit*, stomach, per kg., 2 gm.

Anesthesia: *Fowl*, rectum, per kg., 2 c.c. (Edmunds and Roth, 1908).

Paraphenylenediamin.

Eye Changes: *Dog*, hypodermic, 75 mg. per kg. (Troell, 1916).

Pellotin.

Fatal: *Mouse*, per kg., 68 mg. (Pincussohn, 1907).

Peptone, Witte's.

Temperature: *Rabbit* or *Cat*, hypodermic, per kg., 1 gm. (5 c.c. of 20 per cent.) (Chap. 39, VI).*

Anticoagulant: *Mammals*, vein, per kg., 0.25 to 0.5 gm. (2.5 to 5 c.c. of 10 per cent.).*

Shock: *Mammals*, vein, per kg., 0.2 to 0.5 gm. (2 to 5 c.c. of 10 per cent. (Chap. 43, III, 6).* (Details, Pearce and Eisenbrey, 1910.)

Permanganate, Potassium.

Antidote: *Mammals*, stomach, per kg., 15 c.c. of 1 per cent. (Chap. 40, VI, 1).*

Gastritis: *Rabbit*, stomach, per kg., 0.2 gm. *Dog*, ditto, 0.1 gm.

Fatal: *Rabbit*, stomach, per kg., 0.6 gm. *Dog*, ditto, 0.4 gm.

Peronin.

Respiration: *Rabbit*, hypodermic, per kg., 15 mg.

Phenacetin. See *Acetphenetid.***Phenol.**

Circulation: *Mammals*, vein, per kg., 30 mg. (3 c.c. of 1 per cent.) (Chap. 43, IV, 7)*; stomach, per kg., 1 gm.* (recovery by lavage).

Convulsions: *Frog*, lymph-sac, 10 mg. (1 c.c. of 1 per cent.) (Chap. 32, I, 9).*

M. F. D.: *Cat*, hypodermic, per kg., 0.09 gm. (as 2.5 per cent.). *Rabbit*, hypodermic or stomach, per kg., 0.6 gm. *Guinea-pig*, hypodermic or peritoneum, per kg., 0.25 to 0.5 gm. *White Mouse*, hypodermic, per kg., 0.35 to 0.6 gm. *Frog*, lymph-sac, per gm., 0.1 to 0.6 gm. (as 5 per cent.).

Phenolsulphonephthalein.

Excretion: *Man*, intramuscular, 0.6 mg. (1 c.c. of the solution) (Chap. 15, VII, B).*

Phenylhydrazin Hydrochlorid.

Fatal: *Rabbit*, hypodermic, per kg., 0.14 gm., death in twenty minutes; 0.07 gm., death on second day.

Phlorhizin.

Diabetes: *Mammals*, hypodermic, per kg., 0.3 mg., minimal effect.; 0.15 gm., maximal effect. *Rabbit*, hypodermic, per kg., 0.25 gm., class work (Chap. 39, IX).*

Dog, vein, per kg., 0.1 gm. (not dangerous).*

Phosphate, Sodium.

Harmless: *Dog*, vein, per kg., 35 c.c. of 5 per cent. crystals.*

Phosphorus.

Fatty Liver: *Mammals*, stomach, per kg., 1 to 20 mg., in oil or mucilage. *Frogs*, stomach, $\frac{1}{4}$ to 4 mg. (Details, Abderhalden, 5, 1233.)

Kidney Lesions: Fiske and Karsner, 1914.

Physostigmin Salts.

Circulation and Intestine: *Mammals*, hypodermic, per kg., 0.5 to 2 mg.*

Antidote to Magnesium: *Rabbit*, vein, per kg., 1 mg. (Joseph and Meltzer, 1909).

Muscular Fibrillation: *Rabbit*, vein, per kg., 5 mg. (5 c.c. of 1 : 1000) (Chap. 32, IV, 6).*

Antidote to Curare: *Rabbit*, vein, per kg., 8 mg. (Magnus, 1908).

M. F. D.: *Dog*, hypodermic, per kg., 4 to 5 mg. *Cat*, ditto, 3 mg. *Rabbit*, ditto, 3 mg. *Guinea-pig*, ditto, 5 mg.

Fatal: *Frog*, lymph-sac, 0.5 mg.

Picric Acid.

Fatal: *Rabbit*, vein, per kg., 0.15 gm.; hypodermic, per kg., 0.2 gm.

Picrotoxin.

Just Convulsive: *Dog*, vein, per kg., 0.3 mg.; hypodermic, per kg., 0.75 mg.; stomach, per kg., 2.25 mg. *Guinea-pig*, vein, per kg., 1 mg.; hypodermic, per kg., 5 mg.; stomach, per kg., 50 mg. *Rabbit*, vein, per kg., 1.5 mg.; hypodermic, per kg., 5 mg.; stomach, per kg., 20 mg. (Hatcher and Eggleston, 1914, Jour. Amer. Med. Assoc., 63, 469).

Convulsions: *Cat*, hypodermic, per kg., 1 mg. *Guinea-pig*, hypodermic, per kg., 5 mg. *Frog*, lymph-sac, 6 mg. (1.5 c.c. of 1 : 250) (Chap. 32, I, 8).*

M. F. D.: *Dog*, hypodermic, per kg., 1.5 mg. *Guinea-pig*, hypodermic, per kg., 16 mg.

Fatal: *Frog*, lymph-sac, 10 mg.

Pilocarpin Hydrochlorid.

Systemic Effects: *Mammals*, vein, per kg., 1 mg. ($\frac{1}{10}$ c.c. of 1 per cent.) (Chap. 44, II, 5).* *Mammals*, hypodermic, per kg., 5 mg. (0.5 c.c. of 1 per cent.) (Chap. 37, V, 1).*

Emetic Effects: *Dog*, vein, per kg., 0.7 mg. (Eggleston, 1916).

Bronchial Constriction: *Rabbit*, vein, per kg., 1 mg. (1 c.c. of 1 : 1000) (Chap. 37, VII).*

Peristalsis: *Rabbit*, vein, per kg., 3 mg. (3 c.c. of 1 : 1000) (Chap. 34, I, 8).*

Piperidin.

Systemic Effects: *Dog*, hypodermic, per kg., 20 mg.

Pituitary Solution.

Circulation and Urine: *Mammals*, vein, per kg., 0.1 c.c. (Chap. 43, II, 4).*

Peristalsis: *Rabbit*, vein, per kg., 0.5 c.c. (Chap. 34, I, 9).*

Potassium Chlorid.

Circulation: *Mammals*, vein, per kg., 10 mg. (1 c.c. of 1 per cent.) (Chap. 45, V, 3).*

Reflexes: *Frog*, lymph-sac, 15 mg. (0.3 c.c. of 5 per cent.) (Chap. 32, III, 5).*

Pyridin.

Fatal: *Rabbit*, hypodermic, per kg., 2.5 mg.

Almost Fatal: *Frog*, lymph-sac, 0.1 gm.

Pyrocatechin.

Pressor: *Dog*, vein, per kg., 2 mg.

Convulsions: *Dog*, vein, per kg., 8 mg.*

Pyrogallol.

Fatal: *Dog*, hypodermic, per kg., 0.2 gm.; stomach, per kg., 0.125 gm.

Quinin Hydrochlorid.

Excretion: *Man*, mouth, 0.2 gm.*

Metabolism: *Mammals*, stomach, per kg., 0.05 gm.

Circulation: *Mammals*, vein, per kg., 10 mg. (to 100 mg.).*

Leukocytes: *Frog*, lymph-sac, 1.25 to 10 mg.*

M. F. D.: *Rabbit*, hypodermic, per kg., 0.5 gm. *White Mice*, hypodermic, per kg., 0.7 gm. (Smith and Fantus, 1916). *Pigeon*, hypodermic, per kg., 0.4 gm. *Frog*, hypodermic, per kg., 0.4 gm. (0.35 gm., Smith and Fantus, 1916).

Rhubarb.

Excretion: *Man*, mouth, 1 c.c. of Fldext. (Chap. 15, XII).*

Cathartic: *Dog*, stomach, 5 gm.

Ricin (Merck's).

M. F. D.: *Rabbit*, vein, per kg., 0.03 mg.; hypodermic, per kg., 0.07 mg.

Salicylate, Sodium.

Excretion: *Man*, mouth 1 gm. (Chap. 15, III; also other salicylates).*

Just Emetic: *Cat*, hypodermic, per kg., below 0.6 gm.

Convulsive: *Cat*, hypodermic, per kg., 0.9 to 1.1 gm. *Wild Rat*, ditto, 0.65 to 0.75 gm.

Rabbit, ditto, 1.14 to 1.6 gm.

Surely Fatal: *Cat*, hypodermic, per kg., below 0.9 gm. *Wild Rat*, 0.65 gm. *Rabbit*, ditto, 1.6 gm. (Waddell, 1911, Arch. Inter. Med., 8, 748).

M. F. D.: *Dog*, vein, per kg., 1 gm. *Guinea-pig*, hypodermic, per kg., 2 gm. *Frog*, lymph-sac, per gm., 1 gm. (Blanchier, 1879).

Salol.

Excretion: *Man*, mouth, 0.3 gm. (Chap. 15, III).*

Salts.

Fatal Doses: *Guinea-pigs*, vein, Amberg and Helmholtz, 1915, Jour. Pharmacol., 6, 595.

Salvarsan.

Tonic: *Rabbit*, vein, per kg., 6 to 40 mg.

Albuminuria: *Rabbit*, vein, per kg., 50 mg. *Dog*, ditto, 25 to 50 mg.

Fatal Enteritis: *Rabbit*, vein, per kg., 100 mg. *Dog*, ditto, 50 to 100 mg.

Acute Death: *Rabbit*, vein, per kg., 200 mg. (Kochmann, 1912, Muench. Med. Woch., 59, 18).

Santonin.

(For injection, dissolve in dilute NaOH, or use Sodium Santoninate.)

Excretion: *Man*, mouth, 0.05 gm. (Chap. 15, XI).*

Convulsions and Temperature: *Rabbit*, stomach, per kg., 0.5 gm. (10 c.c. of 5 per cent.) (Chap. 39, III).*

Convulsions: *Dog*, hypodermic, per kg., 0.5 gm.

Fatal: *Cat*, hypodermic, per kg., 1 gm. *Rabbit*, ditto, 2.5 gm.

Sapotoxin.

Fatal: *Mammals*, vein, per kg., 1 to 2 gm. *Cat*, hypodermic, per kg., 40 mg.

Scilla.

Emetic: *Dog*, stomach, per kg., 2 gm.*

Circulation: *Mammals*, hypodermic, per kg., 1 to 10 mg.*

M. F. D.: *Guinea-pig*, hypodermic, per kg., 0.4 gm.* *White Rat*, hypodermic, per kg., 20 gm.* *Frog*, hypodermic, per gm., 0.6 gm. (Chap. 36, III).*

Scillain.

Fatal: *Dog*, per kg., 1 gm.

Scopolamin. See *Morphin-scopolamin*.

Effects: *Frog*, lymph-sac, 10 mg. (1 c.c. of 1 per cent.) (Chap. 32, II, 6).*

Senega.

Emetic: *Dog*, stomach, 5 gm. (Chap. 38, XVI).

Senna.

Excretion: *Man*, mouth, 2 gm. (Chap. 15, XII).

Silicate, Sodium.

M. F. D.: *Mammals*, stomach, per kg., 1.5 to 2 gm.; vein, ditto, 0.07 to 0.3 gm. *Frog*, lymph-sac, 0.025 to 0.1 gm.

Silver Nitrate.

Fever: *Rabbit*, hypodermic, 2 c.c. of 2 per cent.

Solanin.

Fatal: *Rabbit*, per kg., 0.1 gm.

Sparteine Sulphate.

Systemic Effects: *Mammals*, vein, per kg., 5 to 25 mg. ($\frac{1}{2}$ to 2.5 c.c. of 1 per cent.) (Chap. 44, II, 4).*

M. F. D.: *Various animals*, hypodermic, per kg., 0.1 to 0.15 gm. *Rabbit*, vein, per kg., 40 to 60 mg.

Squill. See *Scilla*.**Stovain.**

M. F. D.: *Rabbit*, per kg., hypodermic, 0.18 gm.; peritoneum, 0.03 gm.; vein, 0.025 to 0.05 gm. (Baylac, 1905).

Strophanthin (Amorphous or Kombe).

(1 mg. of Amorphous Strophanthin is about equivalent, in effect, to $\frac{2}{3}$ mg. of Ouabain or 20 mg. of Strophanthus.)

Strophanthus.

Pressor: *Mammals*, vein, per kg., 1 mg. ($\frac{1}{10}$ c.c. of 1 per cent.) (Chap. 43, I, 13).*

Fatal: *Mammals*, vein, per kg., 5 mg. ($\frac{1}{2}$ c.c. of 1 per cent.) (Chap. 44, I, 6).*

M. F. D.: *Cat*, vein, per kg., 3 mg. *Frog*, lymph-sac, per gm., 0.006 mg. (Chap. 36, III). *Rat* and *Rabbit*, Gunn, 1913.

Strychnin Salts: Usual Experimental Doses.

(All doses are per kg.)

Respiratory Stimulant: *Rabbit*, hypodermic, 0.2 mg. (0.2 c.c. of 1 : 1000).

Antagonist to Chloral: *Cat*, hypodermic, per kg., 0.1 mg. (0.1 c.c. of 1 : 1000), repeated (Chap. 39, VII).*

Surely Fatal Dose for Antagonism Experiments: *Cat*, hypodermic, per kg., 0.75 mg.

($\frac{3}{4}$ c.c. of 1 : 1000) (Chap. 40, I)*; stomach, per kg., 1 mg. (1 c.c. of 1 : 1000)

(Chap. 40, II)* *Rabbit*, stomach, per kg., 6 mg. (6 c.c. of 1 : 1000)*; hypo-

dermic, per kg., 0.6 mg. (0.6 c.c. of 1 : 1000).*

Therapeutic Stimulant: *Anesthetized Mammals*, vein, 0.05 mg. ($\frac{1}{20}$ c.c. of 1 : 1000) (Chap. 41, VII, 6).*

Toxic Stimulant: *Anesthetized Mammals*, vein, 0.25 mg. ($\frac{1}{4}$ c.c. of 1 : 1000) (Chap. 41, VII, 7).*

Tetanic: *Anesthetized Mammals*, vein, 0.5 mg. ($\frac{1}{2}$ c.c. of 1 : 1000) (Chap. 43, I, 15).*

Vasomotor Depressant: *Anesthetized Mammals*, vein, 1 mg. (Chap. 43, I, 15).*

Reflexes: *Frog* (leopard), lymph-sac, 0.02 mg. (0.2 c.c. of 1 : 10,000) (Chap. 32, III, 5).*

Tetanus: *Frog* (leopard), lymph-sac, 0.25 mg. ($\frac{1}{4}$ c.c. of 1 : 1000) (Chap. 32, V, 1).*

Strychnin Salts: Effects on Non-anesthetized Animals.

(All doses are mg. per kg. Anesthetized animals require considerably larger doses, varying with the anesthesia.)

	Stomach.	Rectal.	Hypodermic.	Vein.	Other channels.
No perceptible effect:					
<i>Dog</i> :	0.05	0.05		
<i>Rabbit</i> :	0.4	0.2		
Hyperexcitability ("Schreckhaft"):					
<i>Dog</i> :	0.075	0.075	0.075	0.075	Intramuscular, 0.08
<i>Cat</i> :	0.1	0.1	0.08	0.02	
<i>Rabbit</i> :	0.2		Intramuscular, 3.0
<i>Guinea-pig</i> :	40	3.0	0.3	
<i>Pigeon</i> :	0.5		
Just Convulsive:					
<i>Dog</i> :	0.175	0.1 to 0.24	0.1 to 0.24		
<i>Rabbit</i> :	0.57	0.20 to 0.4		
<i>Mouse</i> :	0.615		
<i>Pigeon</i> :	0.5		
<i>Frog</i> :	0.5		
Just Tetanic:					
<i>Dog</i> :	0.47	0.25	0.25		10 to 15 per cent. less than the fatal.
<i>Rabbit</i> :	3.0	0.58	0.4	0.155	
<i>Guinea-pig</i> :	
<i>White Mouse</i> :	0.4		
<i>Frog</i> (Leopard):	1.0 to 1.5		
<i>Toad</i> :	1.6		
Just Fatal:					
<i>Dog</i> :	1.2 to 3.9	2.0	0.35 to 0.75	0.4	Bladder, 5.5
<i>Cat</i> :	{ less than 1.0 }	0.75	{ 0.3 to 0.35 }	
<i>Rabbit</i> :	4.24	0.45 to 0.6	0.36	
<i>Guinea-pig</i> :	4.5 to 4.75		
<i>Mouse</i> :	0.78		
<i>Fowl</i> :	2.0		
<i>Pigeon</i> :	1.67		
<i>Frog</i> :	5.55		
<i>Ring Adder</i> :	23.1		

Sulphate, Sodium.

Diuretic: *Dog*, vein, per kg., 25 c.c. of 2.5 per cent. (dried) (Chap. 46, I, 1).*

Sulphocyanid, Potassium.

M. F. D.: *Pigeon*, hypodermic, per kg., 0.5 to 0.75 gm.

Sulphocyanid, Sodium.

Non-toxic: *Dog*, vein, per kg., 35 c.c. of 1.2 per cent.*

M. F. D.: *Rabbits*, vein, per kg., 0.4 to 0.6 gm. (Corper, 1915).

Suprarenal, Dried.

Pressor: *Mammals*, vein, per kg., 10 c.c. of 1 per cent.*

Tartrate, Sodium.

M. F. D.: *Dog*, vein, per kg., 0.02 gm.

Tetra. See *Beta-tetra-hydronaphthylamin*.**Thallium Salts.**

Fatal: *Dog*, per kg., stomach, 0.5 to 1 gm.; hypodermic, 0.15 gm. *Rabbit*, per kg., stomach, 0.5 gm.; hypodermic or vein, 0.04 to 0.06 gm.

Thebain Nitrate.

Convulsive: *Frog*, lymph-sac, 10 mg. (1 c.c. of 1 per cent.) (Chap. 32, II, 6).

M. F. D.: *Cat*, hypodermic, per kg., 8 mg. (G. H. Mueller, 1908). *Rabbit*, per kg., hypodermic, 21 mg.; vein, 5 to 15 mg.

Theobromin-sodium Acetate (Agurin) or Theobromin-sodium Salicylate (Diuretin).

Diuretic: *Man*, mouth, 2 gm. *Mammals*, vein, per kg., 20 to 50 mg. ($\frac{1}{3}$ to $\frac{1}{2}$ c.c. of 10 per cent.) (Chap. 46, II, 4).* *Rabbit*, stomach, per kg., 0.5 to 1 gm.*

Theophyllin-sodium Acetate (Theocin).

Diuretic: *Mammals*, vein, per kg., 10 mg. (1 c.c. of 1 per cent.) (Chap. 46, III, 4).*

M. F. D.: *Dog*, vein, per kg., 0.1 gm. *Guinea-pig*, ditto, 0.2 gm.

Thiosinamin.

Pleural Effusion: *Mammals*, vein, per kg., 0.13 gm. (Chap. 37, XIII).

Thiosulphate, Sodium ("Hyposulphite").

M. F. D.: *Rabbit*, hypodermic, per kg., 1.5 to 2 gm.

Thorium Nitrate.

Non-toxic: *Dog*, stomach, 25 c.c. of 5 per cent.* *Rabbit*, stomach, per kg., 1 gm.*

Tobacco.

0.02 gm. is about equivalent to 1 mg. of nicotine.

Toluylendiamin.

Fatal Hemolysis. *Dog*, hypodermic, per kg., 40 mg.

Turpentine Oil.

Diuretic: *Dog*, stomach, 1 c.c.

Pleural Effusion: *Dog*, pleura, 1 c.c.

Fatal: *Dog*, stomach, 8 to 30 gm.

Tyramin.

Circulation: *Mammals*, vein, per kg., 2 mg. (0.2 c.c. of 1 per cent.) (Chap. 43, II, 6).*

Uranium Salts.

Hydrops: *Rabbit*, hypodermic, 5 mg. (1 c.c. of $\frac{1}{2}$ per cent.), daily three days (Chap. 39, XX)* (Fleckseder, 1906).

Nephritis: *Dog*, hypodermic, 2 to 15 mg. (MacNider, 1912).

M. F. D. (calculated as metal): hypodermic, mg. per kg.: *Dog*, 1.66; *Cat*, 0.41; *Rabbit*, 0.83; *Rat*, 0.41; *Goat*, 1.66; *Birds*, 40 to 44.

Urea.

Non-toxic: *Dog*, vein, per kg., 35 c.c. of 0.9 per cent. in isotonic NaCl.

Urethane.

Anesthetic: *Rabbit*, per kg.: stomach, 1 gm.; rectum, 0.75 gm.; after *morphin*, stomach, 0.75 gm.; rectum, 0.5 gm. (Chap. 41, TN).* *Cat*, stomach, 0.75 gm. per kg. *Dog*, ditto, 1.5 gm. per kg. *Frog*, lymph-sac, 0.2 gm. (2 c.c. of 10 per cent.) (Chap. 32, TN).*

Hepatic Degeneration: *Rabbit*, rectum, per kg., 0.6 gm.*

Fatal: *Rabbit*, rectum, per kg., 1.0 gm.

Urine, Dog's.

Depressor: *Mammals*, vein, per kg., 3 c.c. (Chap. 44, III, 4).*

Valerian Oil.

Antispasmodic: *Rabbit*, hypodermic, per kg., 0.5 gm. (prevents convulsions when given two hours before Ammonium Carbonate, 0.4 gm. per kg., hypodermic).

Veratrin Sulphate. See also *Cevadin*.

Muscular: *Frog*, lymph-sac, 0.05 mg. (0.5 c.c. of 1 : 10,000) (Chap. 32, I, 10; 33, II).*

Immersion, 1 : 100,000 (Chap. 33, II).*

Convulsions: *Rabbit*, hypodermic, per kg., 2 mg. (2 c.c. of 1 : 1000) (Chap. 40, V).*

Gastric Ulcer: *Rabbit*, stomach, 1 c.c. of 1 per cent. (Chap. 39, XVIII, 4).*

Veratrum Viride.

Vagus Center: *Dog*, vein, per kg., 5 mg. ($\frac{1}{2}$ c.c. of 1 per cent.).*

Convulsive: *Frog*, lymph-sac, 5 mg. (0.5 c.c. of 1 per cent.) (Chap. 32, I, 10).

M. F. D.: *Guinea-pig*, hypodermic, per kg., 45 mg.

Veronal, Sodium.

Circulation: *Mammals*, vein, per kg., 0.2 gm. (2 c.c. of 10 per cent.) (Chap. 43, III, 5).*

M. F. D.: hypodermic, gm. per kg.: *Cat*, 0.3 to 0.35; *Rabbit*, 0.4; *Frog*, 1.5 (Roehmer, 1911).

Yohimbin.

Erection: *Mammals*, hypodermic, per kg., 0.5 mg.

M. F. D.: *Mammals*, hypodermic, per kg., 6.5 mg.

Zinc (Zinc-sodium Pyrophosphate or Zinc Valerate, Calculated as ZnO).

Paralysis: *Frog*, 2 to 12 mg.

M. F. D.: *Rabbit*, hypodermic or vein, per kg., 0.08 to 0.09 gm. *Dog*, vein, per kg., 0.07 to 0.12 gm.

Zinc Sulphate.

Emetic: *Dog*, stomach, 50 c.c. of 1 per cent. *Cat*, ditto, 25 c.c. (Chap. 38, XVI).*

Fatal: *Frog*, lymph-sac, per gm., 1 to 2 mg.

Zygadenus.

M. F. D.: *Rabbit*, hypodermic, per kg., 0.6 gm.*

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